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ARTICLE

Identification of the *SERK* Gene Family in *Paulownia fortunei* and Its Involvement in the Response to Biotic and Abiotic Stresses

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ABSTRACT

Somatic embryogenesis receptor-like kinases (*SERKs*) are receptor-like proteins that contain leucine-rich repeats and are involved in various signaling pathways. This study identified *SERK* family members in the *Paulownia fortunei* genome and analyzed their characteristics and expression profiles using bioinformatics methods. We identified 12 *SERK* genes with relatively conserved gene structures and motifs that were distributed unevenly on eight Paulownia chromosomes. The gene promoters contained various *cis*-acting elements that regulated the expression of the *PfSERK* genes in response to hormones and abiotic stresses. Synteny analysis indicated that 10 segmental duplication events had occurred during evolution of the *PfSERK* family. The expression profile of *PfSERKs* in various tissues of *Paulownia fortunei* was detected by quantitative real-time PCR (qRT-PCR). Many *PfSERK* genes can respond to drought and salt stress. Combined with RNA-seq and protein interaction network, it is speculated that *PfSERK3/11* may participate in the occurrence of Paulownia witches' broom (PaWB) by regulating the plant height of Paulownia.

KEYWORDS

Gene expression; *Paulownia fortunei*; Paulownia witches' broom; *SERK*

1 Introduction

Somatic embryogenesis receptor-like kinases (SERKs) belong to a subfamily of the leucine-rich repeat receptor-like protein kinase (LRR-RLK) family. *SERK* genes generally contain 11 exons that encode seven functional domains in the SERK proteins, namely the SP, ZIP, LRR, SPP, TM, cytoplasmic Ser/Thr kinase domain, and the carboxyl-terminal domain [1,2]. These conserved domains are very important in SERK proteins. For example, ZIP domain is the key domain for the specific binding of SERK [3,4], LRR domain is the key domain for the binding of SERK to plasma membrane [5], and TM domain is the key domain for the separation of intracellular region and extracellular region of SERK proteins [6]. SPP domain is essential in the interaction between protein and cell wall, and is generally considered to be important for the identification of SERKs [3]. *SERK* gene was first identified in carrots in 1997 [7]. Subsequently, SERK homologues were identified in *Arabidopsis*, rice, *Triticum aestivum*, *Malus* × *domestica*, and *Orchidaceae* [3,8–12].

Studies have shown that *SERKs* not only participates in somatic embryogenesis, but also play important roles in disease resistance, hormone signal transduction, and defense response. For example, of the five SERK proteins in *A. thaliana*, AtSERK1 is involved in somatic and zygotic embryogenesis [13]; AtSERK2 is involved in spore formation, differentiation and male gametophyte development [14]; AtSERK3–5 are involved in brassinosteroid (BR) signaling [13–16]. Additionally, overexpression of *AtSERK3* or *AtSERK4* was found to lead to stem elongation [17]. In addition to SERK involvement in the BR pathway, SERK3 also forms a complex with FLS2, another LRR-RLK family protein, that is involved in the innate immune response in plants [18,19]. SERK1, SERK3, and SERK4 participate in regulating the BR signaling pathway, and SERK3 and SERK4 participate in regulating cell death [15,20,21]. Overexpression of *OsSERK1* led to plant dwarfing and *OsSERK2* was associated with the immune response of rice to bacterial blight and rice blast [9,22–24]. Heterologous expression of wheat *TaSERK1–5* in Arabidopsis led to an increased height phenotype [10]. Twelve *SERK* members were identified in apple, among which *MdSERK4*, *MdSERK*6, and *MdSERK10* could respond to salt stress [11]. Previous studies that have focused mainly on *A. thaliana*, *Z. mays*, *O. sativa*, and other model plants, and rarely on tree species with great economic value, such as Paulownia [1,3,8,9]. Economically valuable tree species are important contributors to the economic growth of many countries worldwide. Understanding the role of *SERK* genes involved in the economic tree species will contribute to further research that may lead to breakthroughs in the prevention and control of diseases and pests that help to improve the utilization value of these tree species. Therefore, strengthening the research on growth and development of economic tree species is urgently needed.

With the characteristics of wide adaptation area, fast growth, and good material, Paulownia can play an important role in alleviating wood shortage, ensuring food security and improving the ecological environment [25,26]. However, PaWB disease can causes axillary bud sprouting, shortened internodes, yellowing and smaller leaves, clumping branches, and dwarfing of Paulownia, and leads to the death of young trees and reduction of timber yield [27–29]. In the previous study of our laboratory, it was found that the expression of *PfSERK* changed in phytoplasma-infected *P. fortunei* [28,30]. These findings indicated that *PfSERKs* probably influenced key traits of Paulownia. Understanding the functions of *PfSERKs* will further enrich and improve the understanding of the response of Paulownia to PaWB infection at the molecular level and may help in breeding programs to improve the economic value of Paulownia. We identified the members of *PfSERK* gene family at the whole genome level, and analyzed their physiochemical properties and expression patterns to determine the function of *SERK* in Paulownia.

2 Materials and Methods

2.1 Plant Material

P. fortunei (PF) and PaWB-infected *P. fortunei* (PFI) were used as experimental materials, and the seedling age was 30 days. Paulownia infected with witches' broom disease showed symptoms such as a large number of axillary buds sprouting and clustered branches and leaves. The plantlets were grown based on the method proposed by Fan et al. [31].

2.2 Identification of the P. fortunei PfSERK Gene Family

BlastP search was conducted in the genome database of Paulownia using the known AtSERK1-5 protein sequence, and the candidate protein sequence of PfSERK was screened out (E-value < 1e-5). Further identification of *PfSERK* gene family members using NCBI-CDD (https://www.ncbi.nlm.nih.gov/cdd, accessed on 14 December 2021) and SMART (https://smart.embl.de, accessed on 16 December 2021). ExPASy (https://web.expasy.org/compute_pi/, accessed on 19 January 2022) was used to predicte the physiochemical properties of the PfSERK proteins. The subcellular localization of the PfSERKs was

predicted via the Plant-mPLoc (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/, accessed on 17 March 2022).

2.3 Phylogenetic Analysis

The genome annotation file and the ID file of all the *PfSERKs* were submitted to TBtools to obtain a gene structure map. Construction of phylogenetic tree based on SERK proteins from Paulownia and other five species by MEGA7.0.

2.4 Protein Structure Analysis of PfSERKs

The MEME program (https://meme-suite.org/meme/tools/meme, accessed on 22 June 2022) was applied to identify conserved motifs. The motif number was set to 10. The secondary structures of the PfSERKs were predicted using SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html, accessed on 17 February 2023). PfSERK protein spatial models were developed using SWISS-MODEL (https://swissmodel.expasy.org/interactive, accessed on 06 February 2023).

2.5 Collinearity Analysis of the PfSERKs

The Multiple Collinearity Scan toolkit (MCScanX) was applied for collinearity analysis of *SERK* genes. The Ka/Ks Calculator in TBtools was used for non-synonymous/synonymous (Ka/Ks) analysis of the *PfSERKs*.

2.6 Identification of Cis-Acting Elements

In the region of 2000 bp upstream of *PfSERK* promoters, the *cis*-acting elements were screened by PlantCare (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/, accessed on 02 July 2022).

2.7 Analysis of the Expression Pattern of PfSERKs

Total RNA was extracted from buds, leaves, nodes, internodes, and roots of PF. cDNA was synthesized using StarScript III All-in-one RT Mix with gDNA Remover (GenStar, Beijing), and the 2 × RealStar Fast SYBR qPCR Mix (Low ROX) (GenStar, Beijing) was used for qRT-PCR. The primers of *PfSERK* were designed by NCBI Primer-BLAST (Supplementary Table S1). *PfActin* was applied as the internal reference gene, and there were 3 biological replicates. The $2^{-\Delta\Delta Ct}$ method was applied to determine the expression of *PfSERK* [32,33].

2.8 Analysis of Expression of PfSERKs under Phytoplasma Infection

The transcriptome data of PF, PFI, and 30 mg L-1 Rifampicin (Rif) treated PFI for 15 days and 30 days were downloaded from NCBI database (accession NO, SRR11787938, SRR11787927, SRR11787916, SRR11787905, SRR11787894, SRR11787883, SRR11787960S, RR11787949, SRR11787971, and SRR11787942–SRR11787944) [27,34]. A heat map of *PfSERK* expression levels before and after phytoplasma infection was drawn using TBtools. 4 *PfSERKs* were selected randomly for verification of the transcriptome data by qRT-PCR.

2.9 SERK Protein Interaction Networks

The function of the *SERK* gene in Arabidopsis has been well studied [13–15,17,18]. We used STRING software (http://string-db.org/, accessed on 03 February 2023) to construct SERK protein interaction network using homologous proteins of Arabidopsis.

2.10 Analysis of Expression of PfSERKs under Salt/Drought Stress

Based on the transcriptome data of healthy seedlings of *P. fortunei* treated by drought and salt in our laboratory, we downloaded the transcriptome data from NCBI database (accession number:

PRJNA221355, PRJNA289582, SRP060682). The heat map of *PfSERK* expression levels before and after salt/drought stress was drawn using TBtools.

3 Results

3.1 Identification and Characterization of the PfSERKs

A total of 12 PfSERK protein sequences were obtained by searches of the Paulownia genome using the known AtSERK1–5 protein sequences. The *PfSERKs* were named *PfSERK1–PfSERK12* according to the order of their positions on the chromosomes (Fig. 1). The 12 genes that encode these proteins were distributed on eight Paulownia chromosomes; one was detected on each of chromosomes 2, 4, 7, 11, and 15; two were detected on each of chromosomes 6 and 17; and three were detected on chromosome 13, which had the largest number of *PfSERKs* among all the chromosomes.

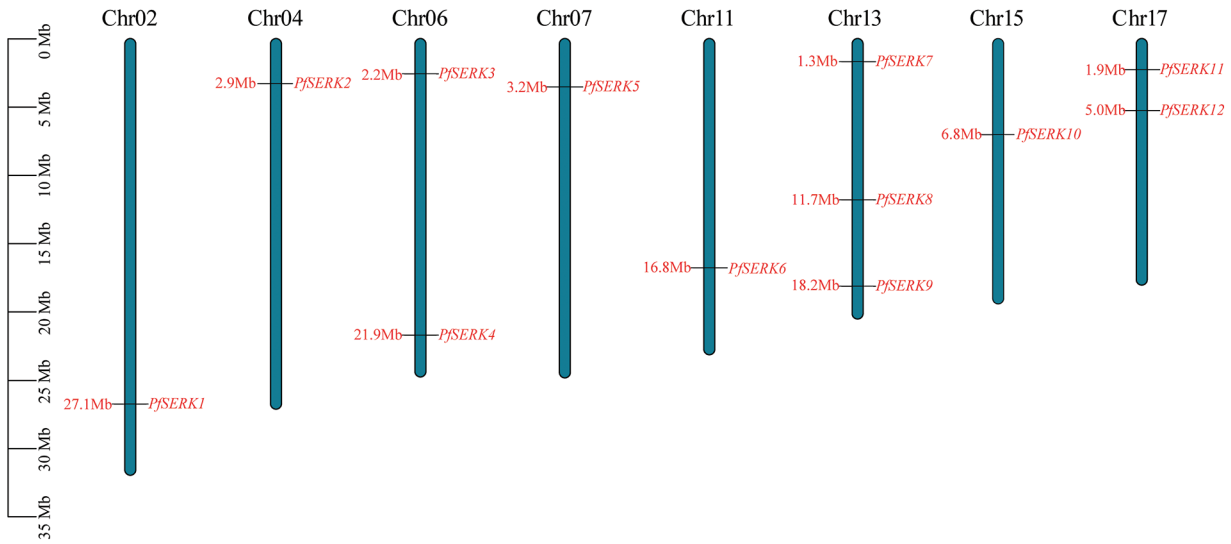

Figure 1: Chromosome distribution of *PfSERK* gene family members

The lengths of the 12 PfSERKs ranged from 588 aa–659 aa; PfSERK6 was the longest and PfSERK7 was the shortest. Their molecular weights ranged from 66230.22 to 72743.65 and their pI ranged from 5.23 to 8.73. Except for PfSERK2, PfSERK4, PfSERK6, PfSERK9, and PfSERK10, the other seven PfSERKs were predicted to be stable (instability index < 40). The predicted results showed that all the 12 PfSERK proteins were hydrophilic proteins, and they were all located on the cell membrane (Table 1).

Table 1: *SERK* gene family information in *P. fortunei*

Gene ID	Gene name	Amino acid	Molecular weight (kD)	pI	Instability Index	GRAVY[a]	Subcellular localization
Pfo02g013940	*PfSERK1*	613	67881.31	5.52	39.43	−0.201	Cell membrane
Pfo04g004050	*PfSERK2*	617	68359.02	5.51	40.13	−0.194	Cell membrane
Pfo06g002260	*PfSERK3*	610	67972.76	5.89	37.89	−0.170	Cell membrane
Pfo06g012410	*PfSERK4*	626	69079.50	7.91	41.65	−0.150	Cell membrane

(Continued)

Table 1 (continued)

Gene ID	Gene name	Amino acid	Molecular weight (kD)	pI	Instability Index	GRAVY[a]	Subcellular localization
Pfo07g004140	*PfSERK5*	623	69091.47	6.61	38.63	−0.134	Cell membrane
Pfo11g008540	*PfSERK6*	659	72743.65	5.23	43.06	−0.053	Cell membrane
Pfo13g001510	*PfSERK7*	588	66230.22	6.02	38.26	−0.084	Cell membrane
Pfo13g004740	*PfSERK8*	620	68976.08	6.50	31.94	−0.085	Cell membrane
Pfo13g009200	*PfSERK9*	627	69306.76	8.73	41.31	−0.148	Cell membrane
Pfo15g008150	*PfSERK10*	628	69341.77	5.66	42.43	−0.138	Cell membrane
Pfo17g002410	*PfSERK11*	623	69225.37	6.51	37.42	−0.153	Cell membrane
Pfo17g006130	*PfSERK12*	625	69486.36	6.62	37.20	−0.204	Cell membrane

3.2 Phylogenetic and Gene Structure Analysis

Phylogenetic analysis of the SERK sequences of *P. fortunei*, *A. thaliana*, *Z. mays*, *O. sativa*, *M. truncatula*, and *Malus* × *domestica* showed that the 12 PfSERKs clustered in three subfamilies (I, II, and III) in the phylogenetic tree (Fig. 2A). Subfamily I had the largest number of proteins, and included all the *A. thaliana*, *Z. mays*, *O. sativa*, and *M. truncatula* SERKs as well as 4 SERKs from *Malus* × *domestica* and 4 SERKs from *P. fortunei*. Subfamily II contained 7 SERKs from *Malus* × *domestica* and 5 SERKs from *P. fortunei*. Subfamily III contained 4 SERKs, one from *Malus* × *domestica* and 3 from *P. fortunei*. Analysis of the gene structure of the *PfSERKs* may be useful for studying their evolutionary relationships. We found that except for *PfSERK7*, which contained 12 exons, the other 11 *PfSERKs* contained 11 exons, and the exons of the *PfSERKs* that shares a close evolutionary relationship were similar in length and position (Fig. 2B).

3.3 Structure of the PfSERK Proteins

The MEME analysis of the conserved domains of the PfSERKs showed that, except for PfSERK3 and PfSERK7, all the other PfSERKs contained 10 conserved motifs that were similar in length and location (Fig. 2B). The predicted secondary structures of PfSERK protein showed that they had similar secondary structures, mainly α-helix (40.35%–46.43%) and random curl (36.07%–40.89%), followed by extended strand (12.14%–14.56%) and β-turn (3.74%–5.90%) (Fig. 3A). The predicted tertiary structures of PfSERK protein showed that they also have similar three-dimensional spatial structure of protein, and some differences are found in their tertiary structures, such as the length of α-helix, β-turn and random coil (Fig. 3B).

3.4 Collinearity Analysis of the PfSERK

Gene duplication events occur during plant genome evolution. We searched for *PfSERK* duplicates and detected 10 segmental duplications among the *PfSERKs*, namely *PfSERK1* and *PfSERK2/6*; *PfSERK3* and *PfSERK7*; *PfSERK4* and *PfSERK5/9/11*; *PfSERK5* and *PfSERK9/11*; *PfSERK6* and *PfSERK10*; and *PfSERK9* and *PfSERK11* (Fig. 4). This finding indicates that amplification of the *PfSERK* gene family was largely influenced by segmental duplication. According to the collinear map of *AtSERKs* and *PfSERKs*, two homologous genes of *SERK*, *PfSERK6* and *AtSERK1/2*, were found (Fig. 5). We calculated the Ka/Ks ratio of the selection pressure of *PfSERKs* (Table 2), the Ka/Ks of all *PfSERK* family members were much smaller than 1, these resuits indicating that they were subject to purifying selection during the evolutionary process.

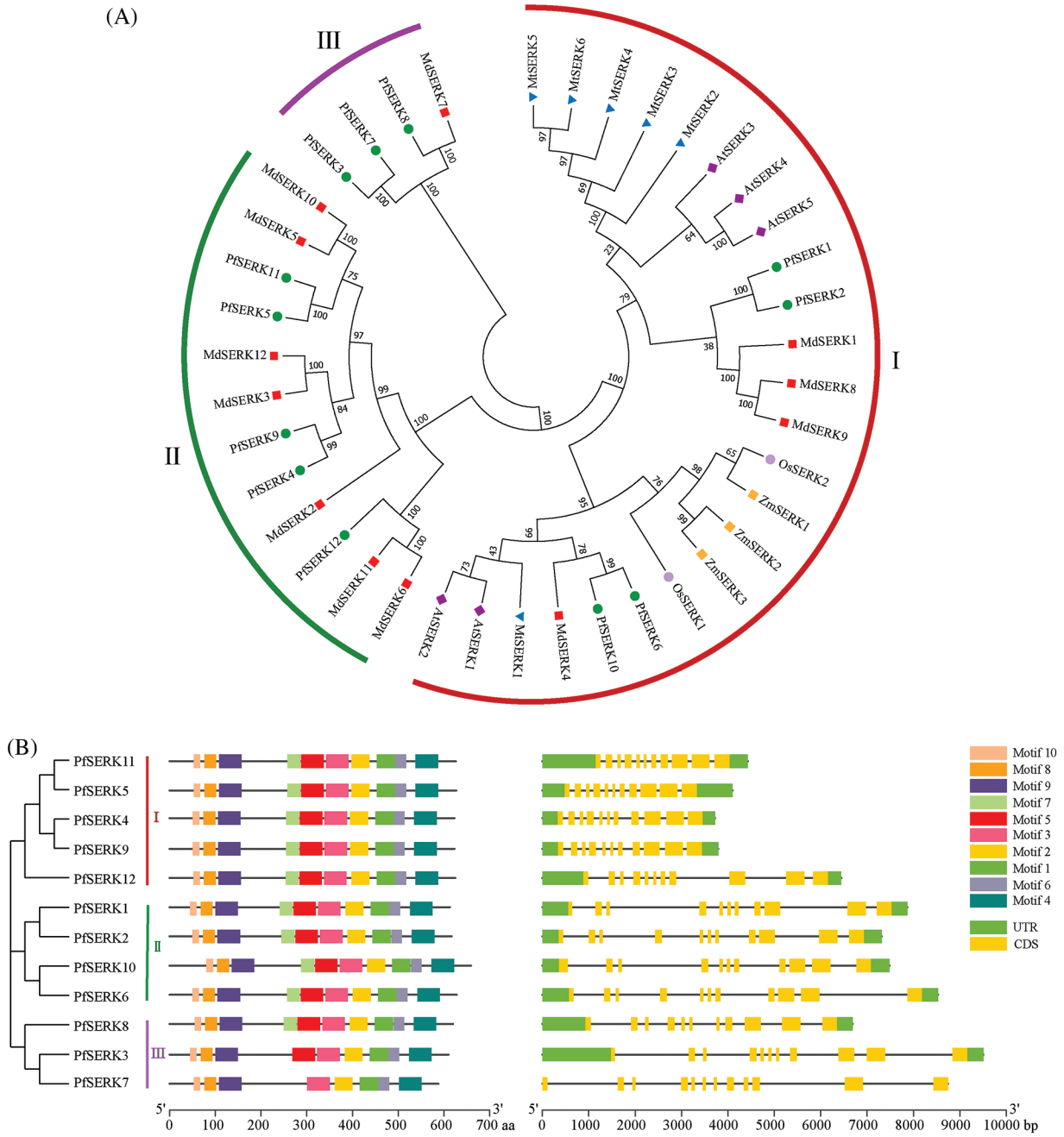

Figure 2: The analysis of phylogenetic, conserved motifs and gene structure. (A) Phylogentic analysis of SERKs from *P. fortunie*, *A. thaliana*, *Z. mays*, *O. sativa*, *M. truncatula*, and *Malus* × *domestice*. (B) The motif composition of PfSERKs (left) and the exon-intron structure of *PfSERKs* (right)

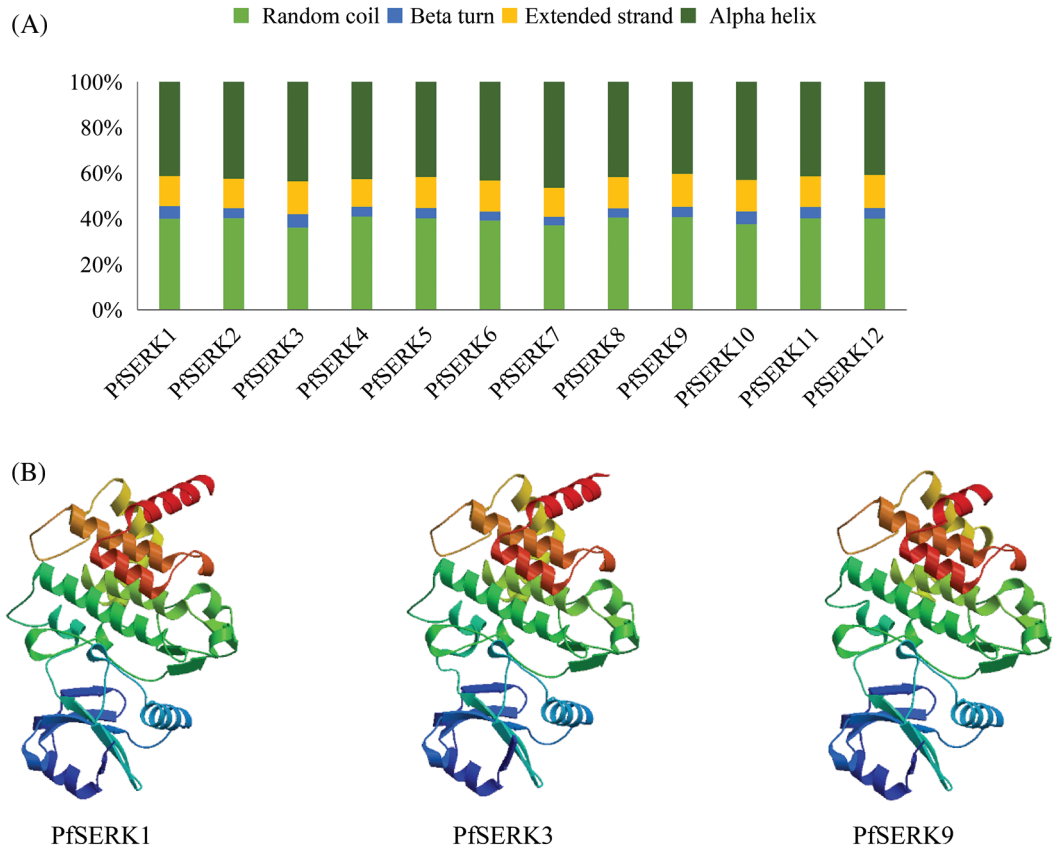

Figure 3: Protein structure of PfSERKs. (A) Secondary structure percentage diagram of PfSERK proteins. (B) Tertiary structure of PfSERK proteins

3.5 Analysis of Cis-Acting Elements

The prediction of *cis*-acting elements showed that light response elements were the most common and made up the largest proportion (Fig. 6). Hormone elements such as abscisic acid and auxin were detected, suggesting that the corresponding *PfSERKs* may affect the growth and development of *P. fortunei* by participating in different hormonal regulatory pathways. Drought, low temperature, anaerobic, and other abiotic stress-related response elements were also detected, suggesting that the corresponding *PfSERKs* can respond to stress.

3.6 Expression of PfSERKs in Different P. fortunei Tissues

Expression profiles of 12 *PfSERKs* in different parts of Paulownia showed that the expression levels of *PfSERK2/4/7/8/9/11/12* were highest in buds, subsequent in leaves, and lowest in roots; the expression level of *PfSERK1* was highest in buds, intermediate in leaves and internodes, and lowest in nodes; *PfSERK3* and *PfSERK6* were the most expressed in buds; *PfSERK5* was expressed more in leaves and roots than in other parts; the expression of *PfSERK10* was the highest in nodes (Fig. 7). The above results showed that *PfSERK* gene was expressed in many tissues of Paulownia, which might affect the growth and development of Paulownia trees.

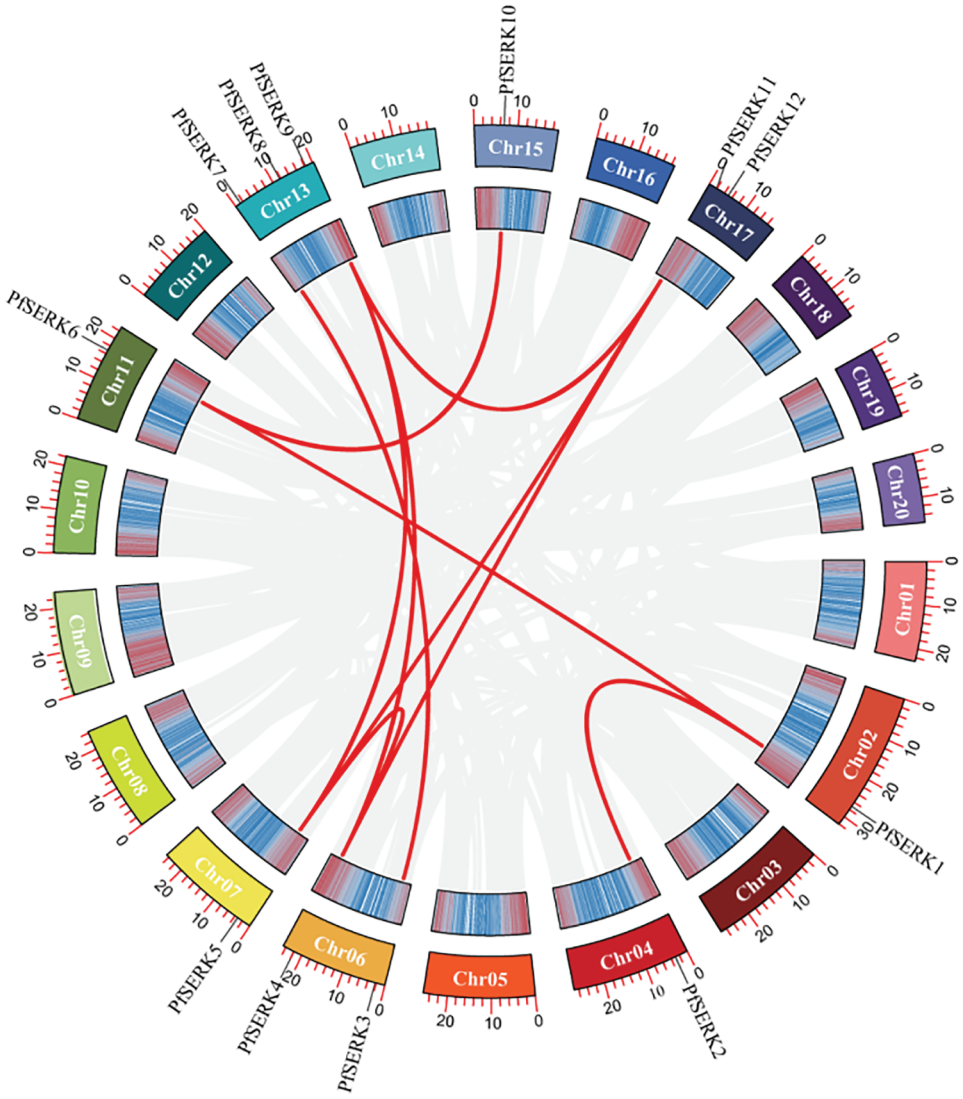

Figure 4: Synteny of *PfSERK* genes. The red lines refer to collinear gene pairs

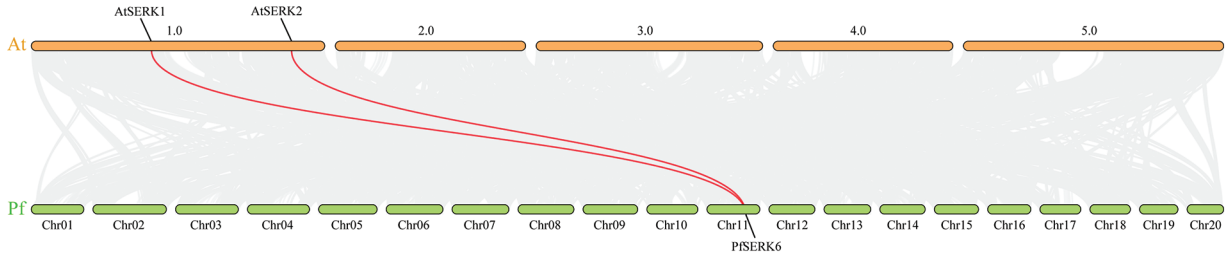

Figure 5: Synteny between *PfSERKs* and *AtSERKs*. The red lines refer to collinear gene pairs

Table 2: Ka/Ks values of *SERK* genes in *P. fortunei*

Gene 1	Gene 2	Ka	Ks	Ka/Ks
PfSERK1	*PfSERK2*	0.023972985	0.345221111	0.069442408
PfSERK1	*PfSERK6*	0.117677175	1.913851564	0.061487096
PfSERK3	*PfSERK7*	0.045913073	0.337590184	0.136002393
PfSERK4	*PfSERK5*	0.156882421	1.615389970	0.097117367
PfSERK4	*PfSERK9*	0.054067720	0.264086580	0.204734826
PfSERK4	*PfSERK11*	0.152170766	1.904421999	0.079903911
PfSERK5	*PfSERK9*	0.155291646	1.861544653	0.083420855
PfSERK5	*PfSERK11*	0.034481999	0.307412003	0.112168681
PfSERK6	*PfSERK10*	0.008770491	0.317186458	0.027650900
PfSERK9	*PfSERK11*	0.151153246	2.028469155	0.074515920

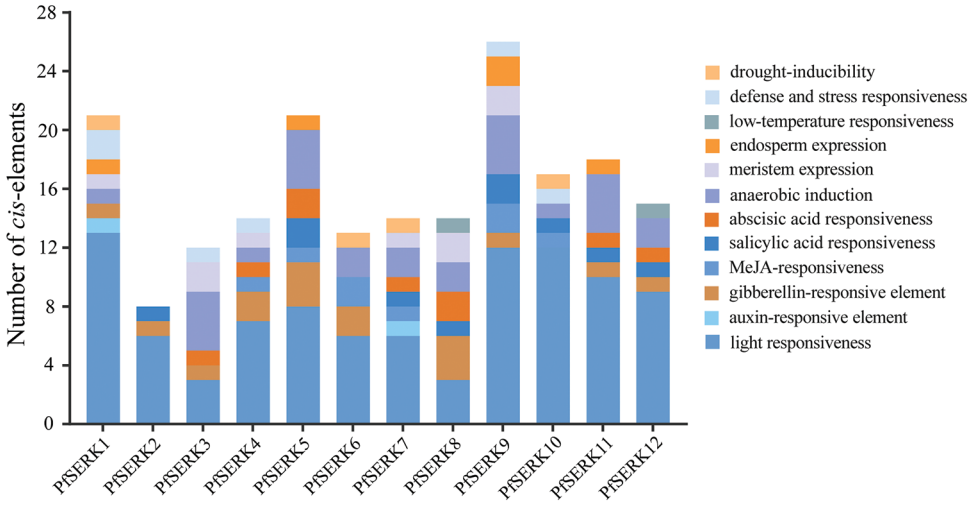

Figure 6: *Cis*-acting elements of *PfSERK* genes

3.7 Analysis of PfSERK Expression under Phytoplasma Infection

To understand the expression pattern of *PfSERKs* with phytoplasma infected, the transcriptome data of the *PfSERKs* in PF and PFI was analyzed. All the *PfSERKs* were expressed in both healthy and PaWB-infected *P. fortunei*. As shown in Fig. 8A, *PfSERK3*, *PfSERK8*, and *PfSERK11* were down-regulated and *PfSERK4*, *PfSERK9*, and *PfSERK12* were up-regulated in the PFI *vs*. PF comparison, it is indicated that they were associated with the occurrence of PaWB.

Furthermore, after treatment with 30 mg L^{-1} Rif, the expression pattern of *PfSERK3* and *PfSERK11* were in accordance with the morphological changes in the simulated system of PaWB (Fig. 8A), indicating that *PfSERK3* and *PfSERK11* may be related to PaWB. qRT-PCR analysis of 4 *PfSERK* genes showed that the gene expression levels were consistent with RNA-seq data, indicating that the transcriptome sequencing data was reliable (Fig. 8B).

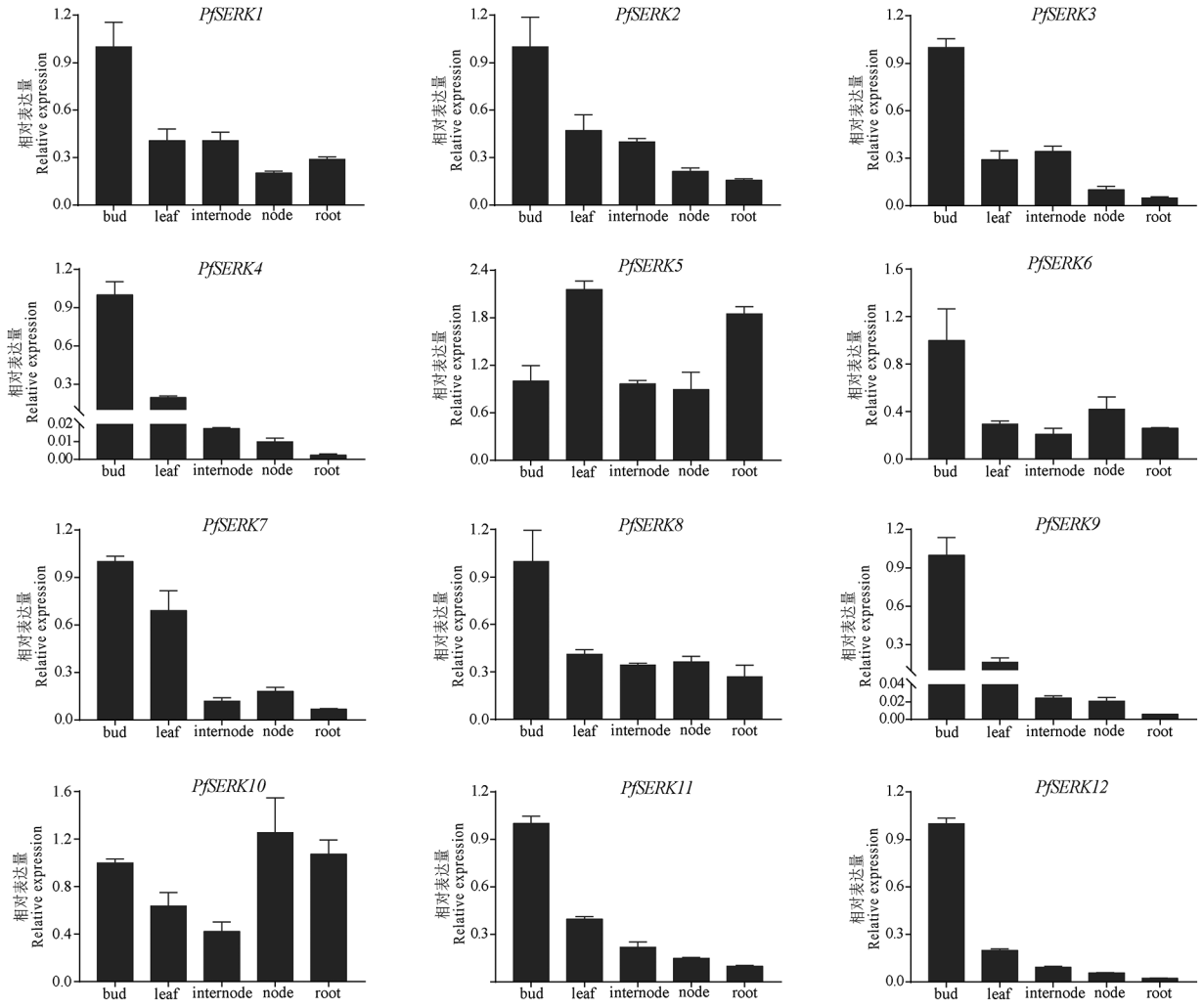

Figure 7: Expression patterns of *PfSERKs* in *P. fortunei*. qRT-PCR analysis of *SERK* gene expression levels in buds, leaves, internodes, nodes, and roots. Bars show mean ± SD (n = 3)

3.8 Interaction Network of PfSERKs and Other Proteins

In order to further explore the function of PfSERK, an interaction PfSERK–protein network was constructed using *A. thaliana* orthologs (Fig. 9). The predicted results show that PfSERK1/3/6 may interact with BRI1, and PfSERK4/6/11/12 may interact with BAK1 and SAC52. It has been proved that BAK1, BRI1 and SAC52 can regulate plant height [15,17,35] so it is speculated that PfSERK1/3/4/6/11/12 may be involved in the regulation of plant height. Further combined with transcriptome data analysis, it was found that *PfSERK3* and *PfSERK11* may associated with the dwarfing of Paulownia witches' broom disease plant.

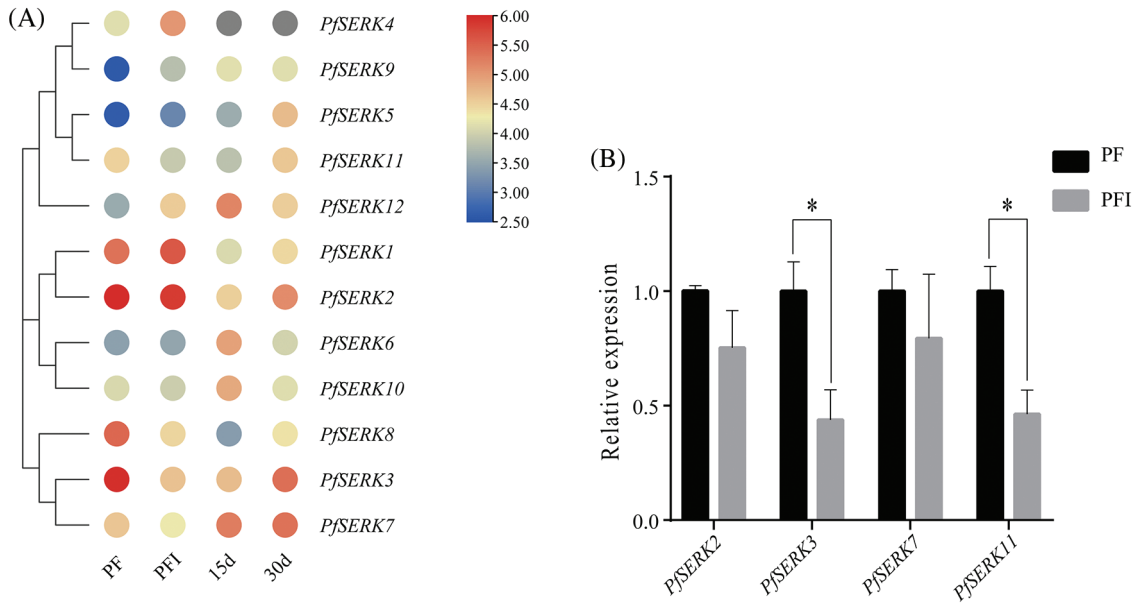

Figure 8: Expression analysis of *PfSERK* genes under phytoplasma infection. (A) *PfSERK* genes expression in PF, PFI and PFI treated with Rif. (B) results of qRT-PCR validation. Bars show mean ± SD ($n = 3$). $p < 0.05$ was marked with asterisk (*)

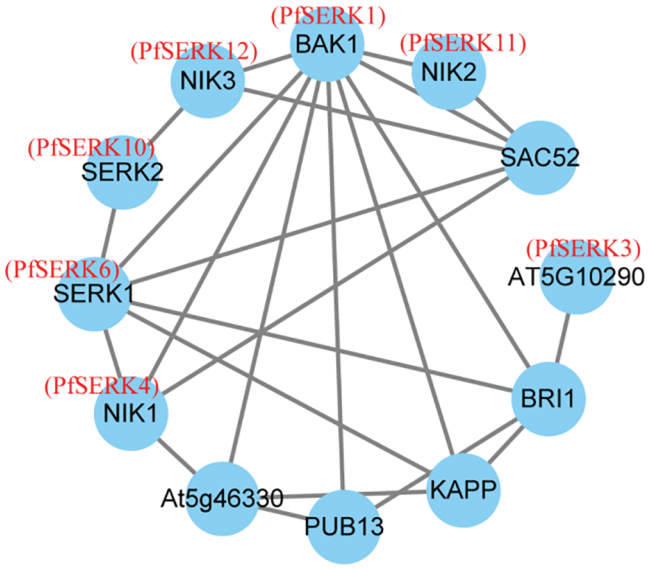

Figure 9: SERK protein interaction network in *P. fortunei*

3.9 Expression of PfSERKs under Drought and Salt Stress

To understand the expression of the *PfSERKs* under abiotic stress, we downloaded and analyzed the RNA-seq data of Paulownia under drought and salt stress (Fig. 10). The expression of *PfSERK1*, *PfSERK2*, *PfSERK4*, *PfSERK5*, *PfSERK8*, *PfSERK9*, and *PfSERK10* were significantly changed after drought treatment of *P. fortunei*. In which, the gene expression level of *PfSERK1* was up-regulated

2 times after drought stress, and the gene expression levels of *PfSERK4*, *PfSERK5*, *PfSERK8*, *PfSERK9*, and *PfSERK10* were significantly down-regulated 2–3 times, indicating that *PfSERK1*, *PfSERK4*, *PfSERK5*, *PfSERK8*, *PfSERK10*, *PfSERK9*, and *PfSERK10* could respond to drought stress of Paulownia. 11 *PfSERK* genes were expressed after control and salt treatment, among them, the expression level of *PfSERK3* changed significantly after salt stress. The gene expression level of *PfSERK3* was down-regulated by 2 times after salt stress, indicating that *PfSERK3* could respond to salt stress of Paulownia.

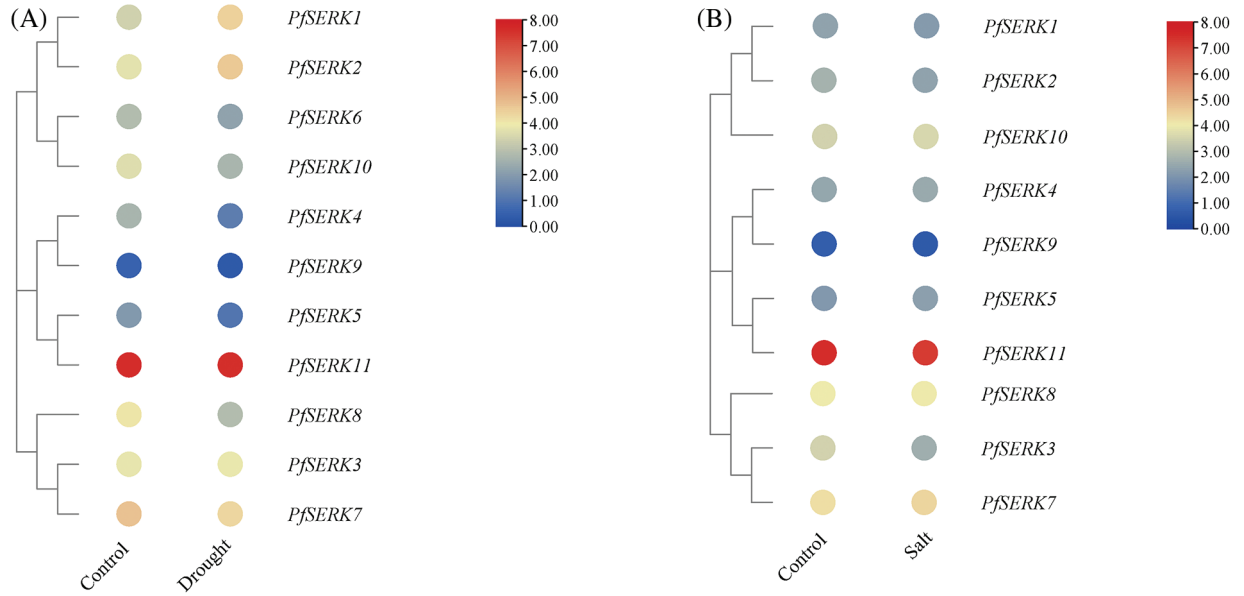

Figure 10: Expression pattern of *PfSERKs* under drought (A) and salt (B) stress

4 Discussion

SERK is a highly conserved class of protein kinases that play regulatory role in the process of stress response [14,36–38]. We identified 12 members of *PfSERK* family, and made a preliminary prediction of their subcellular localization. It was found that these members all located on the cell membrane. The analysis of physiochemical properties of PfSERK found that most PfSERKs were similar in length and molecular weight to AtSERKs, and more than half of PfSERKs were stable. The numbers of exon in the *PfSERKs* was consistent with the numbers found in *SERKs* of other species such as *A. thaliana*, *Malus* × *domestica*, which indicates that *SERKs* were relatively conserved in the evolutionary process of plant species [3,11,24]. The PfSERKs contained similar domains as SERKs in other species, indicating that they had a close evolutionary relationship. The structure of PfSERK protein is highly conserved in α-helix, β-turns, extended strand, and random coil, which indicates that PfSERKs have similar secondary structures, and the function of protein is determined by its structure, so it is speculated that PfSERKs may have similar functions.

The promoters of several *PfSERKs* contained hormone-related and stress-related response elements, indicating that these genes are closely associated with the growth of Paulownia and the relevant response to stress. Gene duplication is important for expanding the number of gene family members and increasing their functional diversity [39,40]. We identified 10 gene pairs among the 12 *PfSERKs* that may have been involved in fragment replication events, and speculated that fragment replication may be very important in *PfSERK* gene expansion. In our research, two *SERK* collinear gene pairs were identified from

P. fortunei and *A. thaliana*, namely, *PfSERK6* was identified as the homologous gene of *AtSERK1* and *AtSERK2*, so it is speculated that *PfSERK6* may have similar functions to *AtSERK1* and *AtSERK2*.

The 12 analyzed *PfSERK* genes were expressed in buds, leaves, internodes, nodes and roots, and the expression levels were different, indicating that the *PfSER*K family may be associated with the Paulownia growth and development. It has been reported that *SERK* can respond to abiotic stress. For example, overexpression of *OsSERK2* can obviously increase the grain size of rice and enhance its salt tolerance [41]; *MdSERK4*, *MdSERK6*, *MdSERK7* can respond to salt stress in apple [11]; *HvSERK1* and *HvSERK3* have regulatory effect on salt tolerance in barley [42]. Multiple *PfSERK* genes were able to respond to drought and salt stress, among which *PfSERK1/4/5/8/9/10* were sensitive to drought stress and only *PfSERK3* was sensitive to salt stress.

The prediction of the PfSERK–protein interaction network showed that PfSERK3 may interact with BRI1, PfSERK11 may interact with SAC52, and BRI1 and SAC52 are both known to be involved in regulating plant height [15,35]. We also found that the expression of *PfSERK3* and *PfSERK11* genes differed significantly before and after the occurrence of PaWB, and they were expressed in nodes and internodes to varying degrees. Combined with the expression patterns of *PfSERK3* and *PfSERK11* and the prediction results of protein interaction, we speculated that *PfSERK3* and *PfSERK11* may be involved in the occurrence of PaWB by regulating the plant height of Paulownia. However, the functions of the *PfSERKs* need to be confirmed experimentally in future research. The functions of the *PfSERKs* can be verified by, for example, transgenic experiments to determine their specific roles in the infection process by PaWB.

5 Conclusion

This is the first time to analyze the *SERK* gene family of *P. fortunei* in the whole genome. We conducted detailed bioinformatics analyses of the *PfSERKs*, including gene chromosome localization, chemical signatures, phylogeny, synteny, gene structure, promoter sequences, and SERK–protein interactions. The expression of *PfSERKs* in different parts of *P. fortune*i and in response to biotic and abiotic stresses was also analyzed. These findings are benefit to further understanding the fuction of *PfSERKs* in Paulownia.

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Conflicts of Interest: The authors declare that they have no conflicts of interest for the present study.

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Supplementary Materials

Table S1: The primers used for qRT-PCR

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
PfSERK1	ATGGATCGTTCAATTGCCGC	AGGATCGGCCAAATTGCTCT
PfSERK2	GCCACGATCTTGTACTTGGGT	ACCAAGTGCAGGGATTGACA
PfSERK3	TGAAGATGCTGACGACGCTC	GATTGCCTACGGAAGACGGT
PfSERK4	TGCTGGCGGCATAACATTGA	GCCCACATCCAACGACAAGT
PfSERK5	AAGACGGGGGTAACATCGGT	GACGCGAAGCAACATTTCCAT
PfSERK6	GAGGCATTATCTCTTGAGCTGGT	CACACCACCAGTGACACCAT
PfSERK7	ATTGCAGCTTGCTACGGACA	CGTGGTGCAAAAG CCAATCA
PfSERK8	AGCATTGGTGCATTCTCCCT	CTTGCGCTCATCTTCACCAG
PfSERK9	AGCCTTGGTTTTATCTGTCTGCT	GGAAACTTCCTCGTGTTGACG
PfSERK10	AGCTAATACAGGTTGCCCTGC	TGCCACTCATCCCATTTCTCA
PfSERK11	GGTCATCAGGAACGCCATCT	TCCACCAGAGCAGAAAACCA
PfSERK12	ATTTGCGGGCAGACTTCTCA	CCACCAAACAAGCAAGCCAA
PfActin	AATGGAATCTGCTGGAAT	ACTGAGGACAATGTTACC