Identification and characteristics of SnRK genes and cold stress-induced expression profiles in Liriodendron chinense

Rongxue Li¹, Yasmina Radani¹, Baseer Ahmad², Ali Movahedi¹* and Liming Yang¹*

Abstract

Background: The sucrose non-fermenting 1 (SNF1)-related protein kinases (SnRKs) play a vivid role in regulating plant metabolism and stress response, providing a pathway for regulation between metabolism and stress signals. Conducting identification and stress response studies on SnRKs in plants contributes to the development of strategies for tree species that are more tolerant to stress conditions.

Results: In the present study, a total of 30 LcSnRKs were identified in Liriodendron chinense (L. chinense) genome, which was distributed across 15 chromosomes and 4 scaffolds. It could be divided into three subfamilies: SnRK1, SnRK2, and SnRK3 based on phylogenetic analysis and domain types. The LcSnRK of the three subfamilies shared the same Ser/Thr kinase structure in gene structure and motif composition, while the functional domains, except for the kinase domain, showed significant differences. A total of 13 collinear gene pairs were detected in L. chinense and Arabidopsis thaliana (A. thaliana), and 18 pairs were detected in L. chinense and rice, suggesting that the LcSnRK family genes may be evolutionarily more closely related to rice. Cis-regulation element analysis showed that LcSnRKs were LTR and TC-rich, which could respond to different environmental stresses. Furthermore, the expression patterns of LcSnRKs are different at different times under low-temperature stress. LcSnRK1s expression tended to be down-regulated under low-temperature stress. The expression of LcSnRK2s tended to be up-regulated under low-temperature stress. The expression trend of LcSnRK3s under low-temperature stress was mainly up-or down-regulated.

Conclusion: The results of this study will provide valuable information for the functional identification of the LcSnRK gene in the future.

Keywords: Cold stress, Expression pattern, Genome-wide identification, L.chinense, SnRK
ubiquitination protein [6]. The KA1 domain can interact with a phosphatase upstream of SnRK1 and other proteins [7–9]. The SnRK2 and SnRK3 subfamilies are plant-specific proteins that have been impressively extended in embryonic plants [10]. An extremely conserved kinase domain was contained in the N-terminal of SnRK2s, and an acidic amino acid fragments domain was contained in the C-terminal of SnRK2s [11]. The C-terminal domain comprises two sub-domains, Domain I and Domain II. Domain I is a feature of all members of the SnRK2 subfamily and must be activated by osmotic stress. Domain II is only specific for ABA-dependent SnRK2s and can eventually respond to ABA [12, 13]. SnRK3 is also known as Calcineurin B-like protein-interacting protein kinase (CIPK) as it can bind to CBL to participate in the Ca2+ mediated stress response. Besides a kinase-conservative domain at the N-terminal, CIPK also had a specific NAF domain that could mediate CBL binding and a PPI-conservative domain that could mediate PP2C binding at the C-terminal [14, 15]. It was concluded that SnRK2 and SnRK3 were initiated from SnRK1 [16] and rapidly extended and separated with plant advancement, taking an interest in the interaction of metabolic direction and stress response signaling pathways, thus conferring multiple effects on terrestrial plants corresponding to the stimulation to the external environment [17].

The plant SnRK family is widely involved in metabolic regulation, plant growth and progress, and abiotic stress response. SnRK1 is the main regulator of cellular energy homeostasis, which is primarily involved in metabolic regulation and affects plant development. SnRK1 can phosphorylate its downstream target, bZIP63, and activate the cytoplasmic pyruvate phosphokinase (cyPPDK) promoter, thereby coordinating the metabolic and developmental mechanisms of A. thaliana seedling [18]. A. thaliana SnRK1 (AtSnRK1) phosphorylates a bZIP63 transcription factor that binds directly to the ARF19 promoter and activates it, thereby regulating lateral root (LR) formation [19]. Among the three SnRK1 genes in A. thaliana, KIN10 (SNF1 kinase homolog 10)/SnRK1.1 and KIN11 (SNF1 kinase homolog 11)/SnRK1.2 play an imperative role since the expression of KIN12/SnRK1.3 cannot be detected in most tissues [20]. Of these, the KIN10 protein can interact with the stomatal development core transcription factor SPEECHLESS (SPCH) and phosphorylate SPCH to improve the protein stability of SPCH and thus promote stomatal development [21]. The protein encoded by members of the SnRK2 subfamily is a monomeric kinase that regulates plant response to various abiotic stresses. Most SnRK2 can be activated by hypertonic stress, except AtSnRK2.9 [12, 22]. SnRK2-PYR/PYL/RCARs-PP2C is the core pathway for plant response to ABA [23]. During the process, the SnRK2 in plants is switched from a quiescent dephosphorylated state to an activated state that is the center reaction of plants response to ABA and natural stresses. Studies revealed that in A. thaliana, the protein kinase of the B2/3 subset of RAF might phosphorylate and induce SnRK 2.2/2.3/2.6. The protein kinase of the B4 subgroup of RAF might phosphorylate and activate the other 6 ABA-independent SnRK2. It reveals the central part of the RAF-SnRK2 cascade pathway in osmotic stress and ABA signaling [2, 3, 24]. ABA-PYL-PP2C is responsible for inhibiting SnRK2 binding release, while RAF mediates SnRK2 self-activation through phosphorylation, thus initiating SnRK activation [4]. AtSnRK2 protein kinase could be activated by drought and ABA treatment and phosphorylate sucrose transport proteins SWEET11 and SWEET12, thereby promoting root growth under drought stress, improving root-shoot ratio, and drought resistance [25]. As part of the calcium signaling pathway, SnRK3 mediates plant development and responses to ionic and nutritional stress such as high sodium levels, potassium deficiency, and nitrogen starvation [5, 26]. When the plant was under high salinity, the generated calcium signal was sensed by SOS3 (AtCBL4) on the cell membrane. Then SOS3 is combined with SOS2 (AtCIPK24) to form a complex that phosphorylates SOS1 (Na+/H+ antiporter) and removes the excess Na+ from the root cells [27, 28]. The CBL1/9-CIPK23 complex could enhance the absorption of environmental potassium by plant cells by activating the potassium channel AKTI [29, 30] and the plasma membrane potassium transporter HAK5 [31, 32]. In vitro recombination experiments showed that the CBL2/3-CIPK3/9/23/26 fractions efficiently triggered the activity of TPK1/3/5, which depended on the cytoplasmic calcium concentration [33]. MdCIPK13 and MdCIPK22 improve salt and drought tolerance by targeting the phosphorylated sucrose transporter MsSUT2.2 in apples [27, 34]. Overexpression of BnCBL1-BnCIPK6 in Brassica napus enhances tolerance to high salinity and low potassium [35]. In addition, CIPK14 also regulates the glucose response and interacts with KIN10/SnRK1.1 and KIN11/SnRK1.2 in A. thaliana [36]. Furthermore, the upstream activated kinases of SnRK1, SnAK1/GRIK1, and SnAK2/GRIK2 phosphorylate and activate SOS2 under salt stress [37]. These results indicate an interaction between members of the SnRK subfamily, which plays a role in plant physiological processes besides their important role in responding to high salinity and drought stress. However, the representation of SnRK family members involved in low-temperature stress response needs further investigation.
As plant genomes are increasingly sequenced and analyzed, the plant SnRK protein kinase family has received ceaseless recognition. Its auxiliary properties and expression profiles in response to natural stresses such as salt, drought, and ABA have been explained [38–40]. In this study, members of the *L. chinense* SnRK (LcSnRK) protein kinase family were analyzed for their structural properties and expression profiles under low-temperature stress. It is grown in southern china and is a vital ornamental species [41]. The low-temperature response genes of *Liriodendron* are expected to be tapped to provide genetic resources and theoretical foundations for the future improvement of *Liriodendron* germplasm through genetic engineering.

## Results

### Documentation and physicochemical properties analysis of LcSnRK

In this study, 30 members of LcSnRKs were identified (Additional file 1: Table S1). Three members of LcSnRKs were categorized into the LcSnRK1 subfamily by Pfam analysis. All delimited the kinase-associated domain KA1 (PF02149), except for two members of LcSnRK1s, which also contained the ubiquitin-related domain (UBA) (PF00627). Therefore, we can further subdivide the members of the LcSnRK1 subfamily without the conservative domain of UBA into SnRK1-A; Members of the LcSnRK1 subfamily with the conservative domain of UBA have been classified as LcSnRK1-B (Fig. 1A). Six LcSnRKs were allocated to the SnRK2 subfamily based on the assessment of the conserved domain I, which is approximately 30 amino acids distant from the kinase domain, except for three members of LcSnRK2s that also contained the domain II. Therefore, we can further subdivide the LcSnRK2 subfamily into SnRK2-A and SnRK2-B according to the presence or absence of domain II. Twenty-one LcSnRKs belong to SnRK3/CIPK subfamily, containing the NAF domain (PF03822). This domain comprises 24 amino acids, which can be combined with CBLs. Based on the conventional domain and its arrangement on the chromosome, 30 members of LcSnRKs have been named LcSnRK1.1 ~ LcSnRK1.3, LcSnRK2.1 ~ LcSnRK2.6, LcSnRK3.1 ~ LcSnRK3.21. The amino acid number distribution of proteins from LcSnRK1s, LcSnRK2s, and LcSnRK3s ranged between 519 ~ 547aa, 265 ~ 355aa, 356 ~ 629aa, respectively. The molecular weight ranges of the proteins of LcSnRK1s, LcSnRK2s, and LcSnRK3s were 58.36 ~ 62.44 kDa, 29.77 ~ 40.95 kDa, and 39.95 ~ 70.77 kDa, respectively.

Of the average length and molecular weight of the proteins within the three LcSnRK subfamilies, the amino acid number (537.7 aa) and molecular weight (61.08 kDa) of LcSnRK1s were the largest, while the amino acid number (301.17 aa) and the molecular weight (34.15 kDa) of LcSnRK2s were the smallest.

According to the analysis of the instability index (I.I.), the proteins of LcSnRK1s were predicted to be unstable; half of the proteins of LcSnRK2s (LcSnRK2.2/2.3/2.6) were stable; however, most proteins of the LcSnRK3s were stable (among them, only the four proteins of LcSnRK3.8/3.9/3.18/3.19 were unstable). Except for the predicted signal peptide in LcSnRK 3.21 (the signal peptide cleavage site is within amino acids 1–25), most LcSnRK proteins lacked the signal peptide. Subcellular localization prediction suggested that all LcSnRKs proteins were localized in the nucleus (Additional file 1: Table S1).

### Phylogenetic relationship of SnRK gene family

To investigate the systemic diversity relationship among altered subfamilies and members of LcSnRK, an NJ phylogenetic tree was generated based on *L. chinense* SnRK protein sequences. It was initiated that LcSnRK was divided into three subfamilies, LcSnRK1, LcSnRK2, and LcSnRK3 (Additional file 2: Fig. S1). Phylogenetic trees constructed using the SnRK family proteins from three species, *A. thaliana*, rice, and *L. chinense*, exhibited a similar number of members in the SnRK1s (three to four) and the SnRK2s (six to ten) that were present in *L. chinense*, as well as *A. thaliana* and rice. Details of the SnRK gene family for rice and *A. thaliana* are presented in Additional file 3: Table S2. Conversely, the number of members of SnRK3s varies by species. For example, 26 are found in *A. thaliana*, 34 in rice, and 21 in *L. chinense* (Fig. 1B).

On the phylogenetic tree, the SnRK genes of each group were distributed equally. Members of the rice SnRK family cluster in the evolutionary branches of the phylogenetic trees outer group. The SnRK1 subfamily has many similarities among its members. Among them, three species had bootstrap scores greater than 50, which may reflect the conservative evolutionary position of LcSnRK1. The bootstrap values of the branches in the SnRK2 and SnRK3 subfamilies are not all greater than 50. Especially in the SnRK2 subfamily where LcSnRK2.4 and *AtSnRK2.4* cluster on the same branch; LcSnRK2.1/2.2/2.3 and *AtSnRK2.7/2.8* were clustered on the same evolutionary branch; LcSnRK2.5/2.6 and *AtSnRK2.2/2.3/2.6* were clustered on the same branch.

### Examination of the gene structure and conserved motif of LcSnRKs

To explore the development of preparing the SnRK family in *L. chinense*, the moderated themes of 30 individuals from the LcSnRK family were analyzed in this consideration (Additional file 4: Fig. S2). Dissimilar subfamilies were found to share common features in motif composition and significant differences (Fig. 2A, B). Motifs 1, 4, and 7 encode a kinase domain in all LcSnRK genes.
Fig. 1 Conserved domains of SnRKs and their phylogenetic relationship. A Distribution of conserved domains in three subfamilies of SnRK. B Phylogenetic tree of SnRK. Green, red, and blue fonts represent members of the AtSnRK, OsSnRK, and LcSnRK families, respectively. Background yellow, blue, and red areas represent SnRK subfamilies 1, 2, and 3, respectively.
Motif 14 only exists in the LcSnRK1 and LcSnRK2 families. Motif 12 is presented in all members except SnRK1-A. Motif 12 in SnRK1-B is arranged at the N-terminal. The arrangement pattern of motifs 12, 5, and 14 only exists in the SnRK2 subfamily. Motifs 10, 11, and 8 encode the NAF conservative domain, so these three motifs only exist in the SnRK3 subfamily. In addition, members of the same subfamily shared similar features in the exon-intron structures of the LcSnRK family genes (Fig. 2A, C). LcSnRK1s contain 10 to 11 introns; LcSnRK2s contain 6 to 9 introns. In contrast, the number of introns varies in the LcSnRK3 subfamily, with 13 members containing three or fewer introns and 8 members containing over 10 introns, which differ from the other two subfamilies. Therefore, LcSnRK3 could be divided into two subgroups according to the number of introns, an intron-rich subgroup, and an intron-deficient subgroup. The characteristics of the number of introns suggest that genes in the SnRK1 and SnRK2 subfamilies are structurally more conservative than genes in the LcSnRK3 subfamily.

Cis-regulation element of LcSnRKs
Considering that the SnRK gene family plays a critical part in many natural forms, the promoter of the LcSnRK gene was analyzed in this study to recognize potential administrative components. Hence, giving clues for a more profound understanding of quality control and its reactions provides distinctive boosts. We analyzed the cis-regulatory elements (CREs) on 2kb of the putative promoters of the LcSnRK gene family members. The detailed distribution of each cis-acting element in the promoter region is presented in Additional file 5: Fig. S3. Here, we grouped the CREs according to response to abiotic and biotic stresses, response to phytohormones, and plant growth and development. The heatmap indicates whether the various elements are over- or under-represented relative to 30 members of the LcSnRK family (Fig. 3). G-Box and ABRE are the most widely distributed, and LcSnRK2.2 has the most G-Box and ABRE elements. In the LcSnRK1 subfamily and the LcSnRK2 subfamily, most members (LcSnRK1.2/1.3/2.1/2.2/2.4/2.5) have more G-Box and ABRE elements (over six). Only four members of the LcSnRK3 subfamily (LcSnRK3.5/3.8/3.15/3.18) had more G-Box and ABRE elements. The 12 LcSnRK genes contained LTR, a cis-acting element associated with a low temperature. LcSnRK3.1 contained three LTRs, and the LcSnRK1 subfamily contained no low-temperature responsive elements. Jasmonates (JA) are involved in various aspects of plant development and are part of the growth-defense trade-off. The 22 LcSnRK genes contained
methyl jasmonate-related regulatory elements, including the CGTCA-motif and TGACG-motif. *LcSnRK2.5* and *LcSnRK3.11* contained most of the CGTCA- and TGACG-motif elements, which are over-represented on the *LcSnRK* promoters.

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### Table 3

| Plant growth and development | Abiotic and biotic stresses | Phytohormone responsive |
|-----------------------------|----------------------------|------------------------|
| RC-element                  | GCN5-motif                 | ARF                  |
| GS-site                     | CAS-motif                  | ABRE                 |
| CAT-class                   | MHS                       | TCAATAR              |
| HD/ZIP3                     | box 4                      | TGTCTC               |
| G-box                       | G-box                      | TGA-box              |
| GTI-motif                   | GTI-motif                  | TATTCT               |
| CREB-motif                  | CREB-motif                | TGTCTCG              |
| TcGATA-motif                | TcGATA-motif              | TGTCTA               |
| LTR                         | MBS                       | AMK                  |
| MBS                         | AMK                       | ABA                  |
| ARE                         | TCA-element                | TGTCTG               |
| TGTCTG                      | TGTCTC-motif              | TGTCTA-motif         |
| TGTCTA                      | TGTCTA-motif              | TGTCTA-motif         |
| TGTCTC                      | TGTCTC-motif              | TGTCTA-motif         |
| TGTCTG                      | TGTCTG-motif              | TGTCTA-motif         |
| TGTCTA                      | TGTCTA-motif              | TGTCTA-motif         |
| TGTCTC                      | TGTCTC-motif              | TGTCTA-motif         |
| TGTCTG                      | TGTCTG-motif              | TGTCTA-motif         |

### Figure 3

Typical cis-regulation element analysis of *LcSnRKs*

Chromosomal location, genomic collinearity, and gene duplication analysis of *LcSnRKs*
Twenty-five members of the 30 *LcSnRK* genes were distributed across 16 chromosomes, and 5 genes (*LcSnRK1.3, LcSnRK3.18, LcSnRK3.19, LcSnRK3.20,*...
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were mapped to non-assembled genomic fragments (Fig. 4). The positions of the LcSnRK family genes on the chromosomes were relatively dispersed. Four LcSnRK3 subfamily groups have been arranged into clusters on various chromosomes, including LcSnRK3.6/LcSnRK3.7, LcSnRK3.11/LcSnRK3.12, LcSnRK3.15/LcSnRK3.16, and LcSnRK3.18/LcSnRK3.19. These four gene clusters are called tandem duplicates, in which the two 200bp fragments produced by duplication are within the same chromosome. The term “segment duplication” refers to the occurrence of two duplicate gene pairs in which fragments are on different chromosomes or are dispersed far apart on the same chromosome [42]. Based on BLAST and MCScanX, 10 segment duplication events were identified in this study, and all occurred between different chromosomes (Fig. 5A). This result demonstrated that segment duplication occasions are vital in enhancing the LcSnRK gene. No duplication events were observed within the LcSnRK1 subfamily, affirming the elevated level of preservation within the LcSnRK1 subfamily.

To explore the homology of the SnRK gene family in L. chinense with that in other species, the homology-collinearity analysis of the SnRK gene in L. chinense, A. thaliana, and rice was performed in this study (Fig. 5). The result showed that 13 LcSnRK genes and AtSnRK genes...
were in a collinear relationship (Fig. 5B), and 18 LcSnRK genes were collinear with Oryza sativa SnRK (OsSnRK) genes (Fig. 5C). Therefore, it has been suggested that these LcSnRK genes might be involved in the evolution of the LcSnRK gene family. To test the effect of evolutionary constraints, Ka, Ks, and Ka/Ks of paralytic homologous and orthologous pairs on the SnRK gene family were calculated. The Ka/Ks range for the 10 segment-duplicated gene pairs was 0.055 to 0.295 (Additional file 6: Table S3), indicating that all duplicated gene pairs for LcSnRK were purified by selection. The Ks of the 13 homologous gene pairs in L. chinense and A. thaliana were all “NaN”, indicating that the sequences of the gene pairs in L. chinense and A. thaliana had a high score of divergences (Additional file 7: Table S4). The Ka/Ks of the homologous gene pairs in most L. chinense and rice were also much smaller than 1 (Additional file 8: Table S5), suggesting that the LcSnRK gene family may have arisen during evolution by purifying selection.

Protein interaction of SnRKs
Exploring the regulatory network of a gene or a gene family is essential to restore the balance between resistance and growth. Therefore, the interaction network of the LcSnRK protein was analyzed based on its homology with A. thaliana (Fig. 6, Additional file 9: Table S6). In the protein-protein interaction network, it was found that KIN10, SnRK2.3, SnRK2.4, SnRK2.5, OST1, CIPK3, CIPK8, SOS2, CIPK12, SIP3, and CIPK23 existed at the center of the interaction network and served as an important hub, which might play a central role in life activities. In the SnRK1 subfamily, KIN10 can interact with the transcription factor FUSCA3 (FUS3) and some disease-causing related genes PRL. There was also an interaction between three members of this SnRK1 subfamily.

In the SnRK2 subfamily, members of SnRK2 can interact with ABI1; SnRK 2.3/SnRK2.4/SnRK2.5/SnRK2.6 (OST1) can interact with ABI2; In addition, SnRK2.3 can interact with ABI5, suggesting that the ABI family may be closely related to the SnRK2 subfamily. Both SnRK2.4 and SnRK2.5 can interact with two members of the BRCA2 family.

Within the SnRK3 subfamily, a common SOS signaling pathway and a CIPK-CBL complex pathway like the CBL3 (calcineurin B-like protein 3) have been associated with CIPK3, CIPK8, CIPK23, CIPK12, and SIP3. CIPK23 and SIP3 can also activate AKT1 on the potassium channel. All three subfamilies of SnRK can interact with the same gene family (e.g., the PP2C protein phosphatase family). There are also interactions between the three subfamilies of SnRK. For example, ABI1 can simultaneously bind to and interact with KIN10 and OST1, affecting the expression of downstream genes (Fig. 6, Additional file 9: Table S6).

Three-dimensional structures of SnRK proteins
Kinases are widely involved in plant signal transduction in response to various types of stress, including the SnRK gene family. Based on previous studies, some key members of the SnRK family, such as KIN10, OST1, and SOS2, play important roles in the stress response. LcSnRK1.3, LcSnRK2.3, and LcSnRK3.1 showed the highest homology to KIN10, OST1, and SOS2, respectively, and were selected to analyze their three-dimensional structures (Additional file 10: Table S7). From the three-dimensional structure of LcSnRK1.3, it contained a kinase domain and a UBA domain (ubiquitin-related domain), and LcSnRK1.3 also contained the KA1 domain at the C-terminus (Fig. 7A; Additional file 11: Fig. S4).
Further modeling and comparing the spatial structure of the LcSnRK1.3 catalytic domain confirmed the significant similarity in the three-dimensional structure between LcSnRK1.3 and KIN10. SnRK2.6/OST1 (Open Stomata 1) was crucial for ABA-induced stomatal closure in response to drought. LcSnRK2.3 contains a conservative kinase domain and a C-terminal regulatory region containing two conservative motifs that form the SnRK2 box. The SnRK2 box forms a single α-helix and runs parallel to the αc-helix (Fig. 7B; Additional file 12: Fig. S5). In addition, like AtOST1, LcSnRK2.3 contains the auto-phosphorylated residues S175 and T176. The

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**Fig. 6** Protein interaction analysis of SnRKs and other proteins. The yellow circles represent the hub gene in the protein-protein interaction network.

**Fig. 7** Three-dimensional structure model of LcSnRK1.3 (A), LcSnRK2.3 (B), LcSnRK3.1 (C).
highest homology (74%) was found between LcSnRK3.1 and AtSOS2 (Fig. 7C; Additional file 13: Fig. S6). Typical catalytic domains of CIPKs were found in LcSnRK3.1, including an N-terminal kinase catalytic domain, a self-inhibitory motif characteristic of NAF, and a protein kinase 2C-binding domain of PPI. The three-dimensional structure of LcSnRK3.1, which activated the ring of LcSnRK3.1 from its active site, was remarkably similar to AtSOS2.

**Expressions of LcSnRKs in response to low-temperature stress**

To understand the responses of LcSnRK family individuals to low-temperature stress, transcriptome information from *L. chinense* elevating under low-temperature stress was selected to analyze the expression design of the LcSnRK quality under low-temperature response (Fig. 8). The low-temperature stress treatment cycle could be roughly divided into continuous up and down-regulation. Expression of 29 LcSnRK genes was detected in the *L. chinense* cryo transcriptome data, except for the lack of expression of LcSnRK3.2. Four genes of the LcSnRK2 subfamily (LcSnRK2.1/2.2/2.4/2.5) and nine genes of the LcSnRK3 subfamily (LcSnRK3.1/3.6/3.8/3.9/3.11/3.14/3.16/3.17/3.19) were continuously up-regulated. The expression of eight genes (LcSnRK2.1/2.2/2.4/2.5/3.8/3.9/3.11/3.16) increased after 1 day of low temperature stress. The expression of five genes (LcSnRK3.1/3.6/3.14/3.17/3.19) increased after 3 days of low-temperature stress. One gene in the LcSnRK1 subfamily (LcSnRK1.1) and five genes in the LcSnRK3 subfamily (LcSnRK3.3/3.4/3.7/3.18/3.20) were continuously down-regulated.

Among them, the expression of four genes decreased after 6 hours of low-temperature stress (LcSnRK3.1/3.7/3.18/3.20), and the expression of two genes decreased after 1 day of low-temperature stress (LcSnRK3.3/3.4). Six genes (LcSnRK1.3/2.3/2.6/3.10/3.12/3.15) were briefly up-and down-regulated, and two genes (LcSnRK1.3/2.3) indicated the highest expression level after 6 hours of low-temperature treatment, followed by the weakness in the expression level.

Four genes (LcSnRK2.6/3.10/3.12/3.15) showed the highest expression level 1 day after low-temperature treatment, while their expression level decreased 3 days later. LcSnRK1.2 expression was stable within 1 day and declined abruptly after 3 days. LcSnRK3.5 expression decreased during the first 6 hours of low-temperature treatment, increased after 6 hours, and decreased to a minimum after 3 days.

To further affirm the expression design of the LcSnRK gene within the over transcriptome investigation, five genes of the LcSnRK2 subfamily (LcSnRK2.1/2.3/2.4/2.5/2.6) and nine genes of the LcSnRK3 subfamily (LcSnRK3.1/3.3.8/3.9/3.11/3.14/3.16/3.17/3.19/3.21) were chosen for qPCR confirmation. qPCR and RNA-seq were found to have similar expression patterns (Fig. 9). The transcriptome expression of these 14 LcSnRK genes and the expression trend of qPCR were up-regulated, and the expression trend of eight genes (LcSnRK2.1/2.4/2.5/3.8/3.9/3.11/3.14/3.16) in the transcriptome was continuously up-regulated, with a wavelike increase in qPCR. Five genes (LcSnRK2.6/3.1/3.17/3.19/3.21) indicated wavy up-regulation in both transcriptome and qPCR. One gene (LcSnRK2.3) showed a wavelike up-regulation in the transcriptome, and expression in qPCR continued to increase.

**Discussion**

The SnRK family plays a vital part in plant response reaction signaling pathways and is a keeper in all eukaryotes. SnRK1 is involved in cell energy sensing, while SnRK2 and SnRK3 play fundamental roles in signaling pathways and regulation of gene expression [43, 44]. In later years, different capacities of SnRK family qualities in plants were slowly found and presented in plants [18, 45–47], suggesting that the SnRK gene has greater importance in plant life activities. In this research, a total of 30 members of the LcSnRK family were identified from the L. chinense genome. All LcSnRK members contain kinase domains classified according to their different domains and functions as members of the LcSnRK1, LcSnRK2, and LcSnRK3 subfamilies. The six members were classified into the LcSnRK2 subfamily, and LcSnRK2 could be further classified into three classes according to protein sequence and function. This corresponded to the classification of the SnRK2 subfamily in A. thaliana [11, 13, 48]. While the first group of SnRK2s (AtSnRK2.1/4/5/9/10) revealed no effects by ABA, the second group of SnRK2s (AtSnRK2.7/8) revealed fewer effects by ABA, and the third group of SnRK2s (AtSnRK2.3/3/6) revealed highly activated by ABA. Complete activation of OST1 (the highly homologous kinase of LcSnRK2.3) requires a closed conformation of the αC-helix and phosphorylation of the activation loop to allow complete alignment of the catalytic residues in the active conformation. A highly acidic ABA box, an unknown mechanism required for kinase activity, is important for mediating the interaction of SnRK2 with protein phosphatase type 2C (PP2Cs) [22, 49]. S175 and T176 in OST1 face the catalytic site and shares no effects by ABA. The second group of SnRK2s (AtSnRK2.7/8) revealed fewer effects by ABA, and the third group of SnRK2s (AtSnRK2.3/3/6) revealed highly activated by ABA. Complete activation of OST1 (the highly homologous kinase of LcSnRK2.3) requires a closed conformation of the αC-helix and phosphorylation of the activation loop to allow complete alignment of the catalytic residues in the active conformation. A highly acidic ABA box, an unknown mechanism required for kinase activity, is important for mediating the interaction of SnRK2 with protein phosphatase type 2C (PP2Cs) [22, 49]. S175 and T176 in OST1 face the catalytic site and thus provide the basis for efficient autophosphorylation of OST1, leading to its full activation [50]. LcSnRK3 shares the highest homology with SOS2 among its 21 family members. Recent studies have shown that CIPK24 (SOS2) has basal activity, unlike other members of the CIPK
Fig. 8  RNA-seq expression pattern of LcSnRK family genes under low-temperature stress
family. At the substrate, the active loop of SOS2 protrudes from the active site, allowing for catalysis. These and other data suggest that securing CIPKs kinase activity requires simultaneous release of the activation loop from the active site and the NAF motif from the nucleotide-binding site [51]. This structure suggests that LcSnRK3.1 is not a self-inhibitory authoritative versicle for CIPKs and represents a compositional highlight of the environment, suggesting that plant homologs may be involved in controlling cytoskeletal structure and function.

Fig. 9  Expression patterns of qPCR of LcSnRK family genes under low-temperature stress. Y-axis represents relative expression, and X-axis represents different time points after stress treatment for expression analysis. The mean standard error measurement (SEM) values of three replicates (n = 3) are shown. Each time point was compared with the control group, and the significance was calculated by one-way ANOVA: * means 0.0001 < p < 0.0005, and ** means p < 0.0001
The SnRK proteins from rice, A. thaliana, and L. chinense were developed on the same phylogenetic tree using the neighbor-joining strategy to analyze their origin and characteristics. The LcSnRKs were divided into three subgroups and clustered with the corresponding subgroups of rice and A. thaliana during phylogeny. Therefore, three SnRK subgroups must be established because the diversity of dicotyledonous and monocotyledonous plants. Conservative structural motif analysis provided information on the conservation of LcSnRK function during evolution. We found five conservative motifs related to the functional domain in the plant SnRK protein. Motif 2 and 4 contained Ser/Thr active site and ATP-binding region, respectively. Motif 12, motif 14, and motif 8 contain specific conservative domains of SnRK1 (KA1), SNRK23 (glutamine – 303 to proline – 318), and SnRK3 (NAF), respectively. Different patterns of exon-intron structures also play an essential role in the evolution and function of different gene families [52]. SnRK3 subfamily differs in gene number and can divide SnRK3 subfamily into two branches according to the number of introns [53, 54].

Similarly, the LcSnRK3 subfamily can be divided into two branches: intron-rich and intron-poor. In rice and A. thaliana, the SnRK3 subfamily was subdivided according to the number of introns, suggesting that an increase or decrease in the number of introns might promote the structural evolution of the SnRK gene family prior to eudicots-monocots differentiation [54]. Colina et al. [44] found that the SnRK family originates from green algae, and almost all members respond to osmotic stress. The intron-poor group first appeared in the seed plants. If seed plants were subjected to huge natural weights amid advancement, the intron-rich population would lose introns and become an intron-less population [44]. Similar exon-intron patterns and arrangements of conservative motifs indicate a close evolutionary relationship within the same subfamily. In addition, Jefferes et al. [55] found that the density of introns of genes that can be rapidly expressed in stress responses is significantly lower. Liu et al. [56] speculated that intron loss might be an evolution of rapid stress adaptation in the early evolution of land plants. They also suggested that the absence of introns may have facilitated more rapid developmental adaptation in terrestrial organisms. Therefore, it is reasonable to speculate that genes in the intron deletion group in the SnRK3 subfamily become more highly expressed under stress.

Genomic duplication occurs throughout plant advancement and often leads to the extension of gene families [57]. The evolution of angiosperms frequently exhibits two major types of gene duplication patterns known as tandem and segment gene duplication [58] and plays a significant role in gene family expansion [59]. This study found 14 homologous gene pairs within the LcSnRK family, counting 10 sets of segment-duplicate genes and 4 sets of gene clusters. In addition, we identified 13 pairs of homologous gene pairs between L. chinense and A. thaliana and 18 pairs between L. chinense and rice. The SnRK2 and SnRK3 subfamilies are plant-specific and arise through duplication of the SnRK1 family [43, 44]. The expansion of the SnRK family is likely because large plants are sessile organisms subject to greater biotic and abiotic pressures than animals [44]. The Ka/Ks ratio of the full-length encoded protein sequences of 14 pairs from collateral homologous gene pairs was much lower than 1, indicating that most genes were effectively purified and selected.

Tissue- and time-specific expression patterns of genes in growing plants often reflect differences in the biological function of gene family members and interactions between related signaling pathways [60, 61]. At the transcriptional level, a total of 21 genes in the LcSnRK family continuously increased or decreased under low-temperature stress. The expression of one member of the LcSnRK1 subfamily decreased with increasing low-temperature stress, while that of four members of the LcSnRK2 subfamily increased with increasing low-temperature stress. The expression of 14 genes of the LcSnRK3 subfamily increased or decreased with increasing low-temperature stress. Regarding evolutionary position, LcSnRK2.5 and LcSnRK2.6 were the closest to OsSAPK8 in rice (Fig. 1B). The rice ossapk8 mutant exhibited slow growth and yellowing of leaves under low temperature treatment [62]. Moreover, from the expression pattern of L. chinense leaves under low temperature treatment, the expression of LcSnRK2.5 reached its highest at 6 hours after low-temperature treatment. It then declined but rose 3 days later. The expression of LcSnRK2.6 reached its peak at 3 days of low temperature treatment. In addition, when Agropyron cristatum AcSnRK2.11 was transferred to tobacco, the transgenic plants indicated stronger cold resistance [63]. LcSnRK3.5/3.6/3.7/3.19 is closer to AtCIPK7 in evolutionary terms (Fig. 1B). In A. thaliana, CIPK7 can interact with CBL1 in vivo and in vitro to play a role in the low-temperature response [64]. Under low-temperature treatment, the expression of LcSnRK3.19 reached its peak 6 hours after low-temperature treatment and then declined but increased after 3 days. In addition, AcCIPK5, a cognate gene of AtCIPK12, positively regulated the cold tolerance of A. thaliana in Ananas comosus (L.) [65]. Consequently, we can conclude that individuals of the SnRK2 and SnRK3 subfamilies are more likely to be interested in the low-temperature response reaction in L. chinense.
Similarly, in the promoter sequence of the *LcSnRK* gene, many cis-acting elements associated with low temperature and other abiotic stresses, such as LTR and TC-rich repeats, were found. These results indicated that the *LcSnRK* gene might respond to abiotic stress, particularly low-temperature stress. In addition, *LcSnRK1.3*, *LcSnRK2.3*, and *LcSnRK3.1* shared high homology with KIN10, OST1, and SOS2, respectively, in *A. thaliana*. The studies on KIN10, OST1, and SOS2 in *A. thaliana* were in-depth [1, 66, 67]. KIN10 is the central integrer of the transcriptional network involved in stress and energy signal transduction in plants [1, 68, 69], which can be associated with other family proteins in reaction to abiotic response in plants. OST1 may be involved in the signaling pathway in plants in response to abiotic stress through phosphorylation [70, 71]. The CBL-CIPK (SnRK3) complex is involved in the signal transduction of various stresses. SOS2 physically interacts with the calcium sensor SOS3 [72] and is activated in a calcium-dependent manner [73]. Therefore, the expression of these three genes under abiotic stress indicates certain regularity based on the transcriptional level expression results. For case, *LcSnRK1.1* expression was persistently up-regulated under low-temperature stress, and *LcSnRK2.3* expression appeared to be a short-term expanding drift under low-temperature stress. *LcSnRK3.1* expression was ceaselessly up-regulated under low-temperature response. These three genes may be essential in studying the *L. chinense* response to low-temperature stress.

**Conclusions**

The genes of the SnRK family play essential roles in signaling pathways such as plant response to biotic and abiotic stresses. In this study, 30 members of the SnRK gene family were identified in *L. chinense* and divided into three typical subfamilies with the same features and obvious differences in gene structure and motif composition. In response to *L. chinense* to low-temperature stress, the 30 members of the SnRK gene family had four expression patterns, and the expression trend of 14 genes was continuously increased while that of 6 genes was decreased. These results will give imperative data for examining the structural characteristics and potential natural capacities of the *LcSnRK* gene in *L. chinense*.

**Materials and methods**

**Documentation and physicochemical properties analysis of *LcSnRKs***

The nucleic acid and protein sequences of *L. chinense* were obtained from the *L. chinense* protein database ([https://hardwoodgenomics.org/](https://hardwoodgenomics.org/)) [74]. Protein sequences from the SnRK family of *A. thaliana* and rice were downloaded from the Phytozome database ([https://phytozome-next.jgi.doe.gov/](https://phytozome-next.jgi.doe.gov/)), and the rice annotation project (RAP) ([https://rapdb.dna.affrc.go.jp/](https://rapdb.dna.affrc.go.jp/)) [76], respectively. Local BLASTP searches were completed using the SnRK protein sequences from *A. thaliana* and rice. The Hidden Markov Model (HMM) and the BLASTP program were used for the preliminary identification of *LcSnRK* family proteins. *LcSnRK* candidate proteins were further validated by the NCBI conservative domain database, the SMART database, and the Pfam database ([http://pfam.xfam.org/](http://pfam.xfam.org/)) for domain search [77]. Based on the information in the above three databases, the *LcSnRK* gene with the traditional domain was manually screened. The online software Protparam in the Expasy database ([https://web.expasy.org/protparam/](https://web.expasy.org/protparam/)) was used to identify sequences of *SnRK* of *L. chinense*, the physicochemical properties (including molecular weight, isoelectric point, and hydrophilicity) [78]. Signal peptides of the *LcSnRK* protein were predicted using SignalP ([https://servies.healthtech.dtu.dk/service.php?SignalP](https://servies.healthtech.dtu.dk/service.php?SignalP)). Subcellular localization of the *LcSnRK* protein was predicted by Plant-mPLoc ([http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/](http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/)) [79].

**Phylogenetic analysis of *LcSnRKs***

Phylogenetic analysis was performed using the SnRK members from *L. chinense*, Rice, and *A. thaliana*. The multiple sequences were aligned using ClustalX. The phylogenetic tree was assembled using the NJ method in the MEGA7.0 software [80]. Evolutionary distances were obtained using the p-distance method, which was used to estimate the number of amino acids in each locus. One thousand bootstrap sampling iterations guaranteed the reliability of each phylogenetic tree. iTOL was used for further beautification.

**Chromosome location and gene duplication analysis of *LcSnRKs***

According to the physical location information in the *L. chinense* genome database, the identified *LcSnRK* gene was chromosomally localized using the biological software TBtools [81]. To analyze the duplication events of the *LcSnRK* gene, all *L. chinense* genomic sequences were aligned with BLASTP. The duplication modes of SnRK were divided into segment duplication and tandem duplication using MScanX [82]. Finally, the collinear analysis diagram was constructed using the Circos software [83]. Nonsynonymous (Ka) and synonymous (Ks) substitutions between pairs of interest were calculated using the KaKs_Calculator [84].
Gene structure, conservative motif, and cis-regulation elements of LcSnRKs

The intron-exon structures of the LcSnRKs were mapped onto the diagrams using the annotation files in the L. chinense genome database by TBtools software. The conserved motifs of the SnRK protein in L. chinense were analyzed online by MEME (https://meme-suite.org/meme/doc/meme.html) using the predicted full-length protein sequences of the LcSnRKs. Extracting an upstream sequence (2000 bp) of an initiation codon of each LcSnRK gene from L. chinense genomic sequence. PlantCare (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) [85] was then used to predict the distribution of cis-acting elements in the promoter region of LcSnRKs.

Protein-protein interaction network prediction of SnRKs

Based on the high homology between LcSnRKs and AtSnRKs proteins, a String database (https://cn.string-db.org/) was used to generate a functional protein interaction network. The protein interaction network of the SnRK family was plotted using Cytoscape 3.8.2 [86].

Three-dimensional structure modeling and verification of LcSnRK proteins

The full-length atomic structure of the LcSnRK protein was assembled by the Robetta program (https://robetta.bakerlab.org/) to construct the structure of the protein. The structure of the candidate protein was further explored since the reliability of its protein structure was determined using the online website SAVESv6.0 (https://saves.mbi.ucla.edu/) including ERRAT, PROVE, Ramachandran. Then the 3D modeling was performed using VMD software.

Expression analysis of LcSnRKs based on transcriptome and qRT-PCR

The expression of LcSnRK in response to cold stress was analyzed using the methods of Guan et al. [87] and Li et al. [88] and momentarily attenu. Two-month-old and identically growing Liriodendron hybrid seedlings regenerated from somatic embryos were transferred to an incubator (25°C, 16h light, and 8h dark) for culture for 1 week and then transferred to a 4°C incubator for low-temperature stress treatment. Leaves were detached at 0h, 6h, 24h, and 3 days after cold stress treatment for transcriptome sequencing and qRT-PCR analysis. The RNA was extracted using FastPure® Plant Total RNA Isolation Kit (Polysaccharides&Polyphenolics-rich) (Vazyme, Nanjing, China). RNA purity was checked using the NanoPhotometer® spectrophotometer (IMPLEN, CA, USA). RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA). The library preparations were sequenced on an Illumina Novaseq 6000 platform, and 150bp paired-end reads were generated [89]. Three replicates were set for each independent experiment. The housekeeping L. chinense 18S gene was used as an internal control. All qRT-PCR primers were designed by Primer5.0 (Additional file 14: Table S8). All data generated from real-time PCR amplification was analyzed using a $2^{-\Delta\Delta CT}$ method.

Abbreviations

SnRK: The sucrose non-fermenting 1 (SNF1)-related protein kinases; KA1: Kinase associated-1 domain; UBA: Ubiquitin-associated domain; NAF: National Arbitration Forum Domain; KIN11: SNF1 kinase homolog 11 protein kinase; KIN10: SNF1 kinase homolog 10 protein kinase; OST1: OPEN STOMATA 1 protein kinase; CIPK: Calcineurin B like protein-interacting protein kinase; SOS2: SALT OVERLY SENSITIVE 2 protein kinase; bZIP63: BASIC LEUCINE ZIPPER transcription factor; ABA: Abscisic acid; qRT-PCR: Quantitative real-time polymerase chain reaction.

Supplementary Information

The online version contains supplementary material available at https://doi.org.org/10.1186/s12864-022-08902-0.

Additional file 1: Table S1. Basic protein information of LcSnRK family members.

Additional file 2: Fig. S1. Phylogenetic tree of LcSnRKs. The scale of the length of each branch is labeled at the bottom of the graph, and the bootstrap value of each branch is labeled at the node position.

Additional file 3: Table S2. Details of the SnRK gene family for rice and Arabidopsis.

Additional file 4: Fig. S2. 15 Conservative motifs logo of LcSnRKs.

Additional file 5: Fig. S3. The detailed distribution of each cis-acting element in the promoter region.

Additional file 6: Table S3. The segmental and tandem duplication events of LcSnRKs.

Additional file 7: Table S4. The Ka/Ks ratios between L. chinense and Arabidopsis thaliana.

Additional file 8: Table S5. The Ka/Ks ratios between L. chinense and Oryza sativa.

Additional file 9: Table S6. Detailed information of interaction network of LcSnRKs with other proteins.

Additional file 10: Table S7. Alignment of protein sequences of LcSnRK and AtSnRK family members.

Additional file 11: Fig. S4. Sequence alignment of AKIN10 and LcSnRK1.3 protein. Conservative sites in the activation loop are indicated by a red asterisk, and UBA and KA1 are conservative domains in the SnRK1 subfamily, respectively. Differently colored line segments highlight completely conservative and potential phosphorylated residues.

Additional file 12: Fig. S5. Sequence alignment of AOST1 and LcSnRK2.3 protein. Conservative sites in the activation loop are indicated by a red asterisk. Differently colored line segments highlight completely conservative and potential phosphorylated residues.

Additional file 13: Fig. S6. Sequence alignment of AOST2 and LcSnRK3.1 proteins. Differently colored line segments highlight completely conservative and potential phosphorylated residues.

Additional file 14: Table S8. The primers used in the qRT-PCR.
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Authors’ contributions
LY designed the research. RL conducted experiments. RL and RY contributed analytic tools and manuscript writing. LY, AM, and BA manuscript revision and editing. LY source funding. All authors read and approved the manuscript.

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Availability of data and materials
The datasets generated and/or analysed during the current study are available in the (NCBI website) repository, [https://www.ncbi.nlm.nih.gov/assembly/GCA_003013855.2]. The qRT-PCR data supporting the gene relative expression results of this study can be found in Additional file 14. The transcriptome data in the [NCBI website] repository, [https://www.ncbi.nlm.nih.gov/assembly/GCA_003013855.2]. The qRT-PCR data supporting the gene relative expression results of this study can be found in Additional file 14. The transcriptome data used in this study has been archived and can also be obtained on the NCBI website. The cold stress accession numbers were PRINIA679089 (https://www.ncbi.nlm.nih.gov/bioproject/PRINIA679089/).

Declarations

Ethics approval and consent to participate
The Liriodendron hybrid seedlings regenerated from somatic embryos were grown and collected in the greenhouse in Nanjing Forestry University (Nanjing, Jiangsu province, China). All samples from the cultivar were adopted for all experiments. These plant materials do not include any wild species at risk of extinction. No specific permits are required for sample collection in this study. We comply with the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1 College of Biology and the Environment, Nanjing Forestry University, Nanjing, 210037, China. 2 Muhammad Nawaz Sharif University of Agriculture, Multan, Punjab 5000, Pakistan.

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