Genome-wide identification and expression profiling of the PIN auxin transporter gene family in L. chinense

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Abstract

Background

The genus *Liriodendron* is ancient and contains only two species, *L. chinense* and *L. tulipifera*. These two *Liriodendron* sister species, with a typical intercontinental discontinuous distribution in east Asia (*L. chinense*) and eastern North America (*L. tulipifera*), have great scientific value for paleobotany systematics. *L. chinense* is now recognized as an endangered species partially due to its low natural settling rate. In order to improve our understanding of how this species develops and grows and contribute to protecting this valuable relict species from extinction, it is necessary to explore the mechanisms underlying organ morphogenesis and embryonic development, in which auxin plays an important role. The auxin efflux carrier PIN-FORMED (PIN) proteins are required for the polar transport of auxin between cells through their asymmetric distribution on the plasma membrane, thus mediating the differential distribution of auxin in plants and, finally, affecting plant growth and developmental processes.

Results

In this study, 11 *PIN* genes were identified in the *L. chinense* genome. The structural characteristics and evolutionary status of *LcPIN* genes were thoroughly investigated and interpreted combining physicochemical property analysis, evolutionary analysis, gene structure analysis, chromosomal localization, etc. In addition, motif sequences were used to predict possible functional sites. Further qRT-PCR experiments and transcriptome data analysis indicated that *LcPIN* genes may potentially play an important role during organ development and somatic embryogenesis in *Liriodendron*. For example, specific expression of *LcPIN3* and *LcPIN6a* at different developmental stages of stamens and petals suggests their involvement in the development of these organs.

Conclusion

This study provides a foundation for further genetic and functional analyses of PIN-mediated auxin patterning during organ morphogenesis and embryogenesis in *L. chinense*.

Background

The phytohormone auxin, indole-3-acetic acid (IAA), is a positional signal molecule synthesized within the plant via several different pathways and plays an essential role in regulating various plant growth and developmental processes: embryonic apical-basal polarity establishment, apical and axillary meristem formation, fruit ripening and root architecture construction, as well as plant tropisms such as phototropism, gravitropism and hydrotropism. The transport of polar auxin is mainly performed by plasma membrane auxin transporters which achieve its asymmetric distribution in plants [1–9]. The influx and efflux carriers involved in moving auxin between plant cells are mainly controlled by three gene families: the auxin permease 1 (AUX1)/LAX influx carriers, P-glycoprotein (MDR/PGP/ABCB)

efflux/conditional transporters and the PIN-FORMED(PIN) efflux carriers. *PIN* genes form the most prominent group of auxin carriers by directly controlling auxin polarity distribution, while having coordinated interaction with other gene families [10–13].

The characteristics of asymmetric distribution of *PIN* proteins in cells are particularly compatible with the chemiosmotic hypothesis [14]. Based on this hypothesis, *PIN1* was proposed as an auxin efflux carrier in the *A. thaliana* shoot [15]. Further research identified a total of eight Arabidopsis *PIN* genes, which could be divided into two groups [1]. Five *PIN* proteins (*PIN1-4, PIN7*) with long loops in the hydrophilic domain of the protein are located at the plasma membrane in an asymmetrical distribution and play a pivotal role in cell-to-cell auxin transport [16, 17]. The remaining three PIN proteins (PIN5/6/8) with short loops, are localized at the endoplasmic reticulum membrane. These three *PINs* are proposed to maintain intracellular regulation of auxin homeostasis by working together with the PIN-LIKE auxin efflux carriers [18–21]. Structurally, all *PINs* are membrane proteins. Existing studies have shown that conserved transmembrane domains at the N and C terminus, as well as a central hydrophilic loop region of variable length, influence protein polarity localization patterns and activity of different *PINs* [22, 23]. With the release of genome data for different species, the *PIN* gene family so far has been identified in a variety of living multicellular plants, such as rice, maize, cotton, soybeans, etc. [24–27]. Transcriptome data analysis and qRT-PCR showed that there might be functional interactions and redundancy between *PIN* genes, which also play a role in abiotic stress response and interaction with other plant hormones [27, 28].

The ancient relic plant *Liriodendron* belongs to the *Magnoliaceae* family and occupies a critical evolutionary position. Most of the *Liriodendron* genus went extinct during the Pleistocene, with only two extant relict species remaining to this day: *L. chinense* and *L. tulipifera*. With the completion of the *L. chinense* genome sequence, revealing that *magnoliids* arose before the divergence of eudicots and monocots [29], a basis for further genetic research on *L. chinense* has been provided. Despite the extreme importance of *PINs*, the origins, characteristics and functions of these proteins in *L. chinense* are still largely unknown. In this work, we identify the 11 members of the *PIN* gene family in *L. chinense* and analyze their several properties, including physical and chemical property analysis, evolutionary analysis and gene location. In addition, through multiple sequence alignment of *PIN* protein sequences taken from 17 land plants, we identified conserved functional sites and found that *LcPINs* evolved when dicotyledonous plants diverged. In combination with quantitative tests and transcriptome data analysis we could show that *LcPINs* have different degrees of expression during *Liriodendron* somatic embryogenesis and organ development.

Our study provides a systematical exposition about the evolutionary relationship and structural conservation of *LcPIN* genes, laying a foundation for further functional research.

Results

Genome-wide identification of *PIN* proteins in *L. chinense*

To identify *L. chinense PIN* genes, we used their HMMER and pfam number (PF03547) to search for PIN protein sequences in the *L. chinense* protein database. A local BLASTP algorithm was used with each of the eight *AtPIN* genes as queries. Then, the conserved domain of each candidate gene was predicted using the SMART database. We identified 11 *LcPIN* genes, with two pair of protein sequences (*Lchi22082*/ *Lchi33830* and *Lchi23130/23125*) having a similarity of 100%. Basic gene information, such as gene number, gene location, isoelectric point (pIs) and molecular weight (MW) for the *L. chinense PIN* proteins is listed in Table 1. The identified *LcPIN* encoded proteins range from 233 (*Lchi23130/23125*) to 662 (*Lchi17800*) amino acids in length, with pIs varying from 6.37 (*Lchi15751*) to 9.49 (*Lchi05137*) and MWs varying from 25.04kD (*Lchi23130/23125*) to 71.7kD (*Lchi17800*). The 11 *LcPIN* genes are distributed over 6 chromosomes. Chromosomes 5 and 11 each contain three *LcPIN* genes, while chromosomes 2, 3, 6, 7 and 17 each contain a single *LcPIN* gene (Fig 2A).

Table1: Summary of PIN gene family numbers in *L. chinense*

Gene Name	Gene ID	Locus	Num. of Amino Acids	MW(kDa)	PI
LcPIN1a	Lchi15751	Chr5	608	66.45	6.37
LcPIN1b	Lchi16330	Chr7	623	67.96	9.18
LcPIN1c	Lchi17800	Chr5	662	71.71	8.72
LcPIN2	Lchi05137	Chr17	616	66.77	9.49
LcPIN3	Lchi05662	Chr6	638	69.41	7.69
LcPIN5a-1	Lchi22082	Chr11	358	38.87	7.64
LcPIN5a-2	Lchi33830	Chr3	358	38.87	7.64
LcPIN6a	Lchi02600	Chr5	412	45.59	8.75
LcPIN6b	Lchi01817	Chr2	450	48.66	6.39
LcPIN8a-1	Lchi23125	Chr11	233	25.04	8.55
LcPIN8a-2	Lchi23130	Chr11	233	25.04	8.55

Phylogenetic analysis of the *LcPIN* gene family

To understand the phylogenetic position of the *LcPIN* gene family with respect to *PIN* genes from different plant species, we constructed an evolutionary relationship tree using the *PIN* protein sequences of *L. chinense (Lc), A. trichopoda（Atr）, A. thaliana (At), Z. mays (Zm), O. sativa (Os). V. vinifera (Vv)* and *S. bicolor (Sb)*, using MEGA7.0 with the neighbor-joining algorithm (Fig. 1 & Additional file 4). The *L. chinense PIN* family genes are divided into long (*PIN1, PIN2, PIN3*) and short (*PIN5, PIN6, PIN8*) *PINs* (Table 2). We found that *PIN* genes can be divided into 8 subfamilies: *PIN1, PIN2, PIN3/PIN4/PIN7, PIN10, PIN6, PIN8, PIN5* and *PIN9* (Fig 1). The *PIN1* subgroup has extensively expanded in our selected species (including *L. chinense,* yet excluding *A. thaliana*), suggesting that it may play an important role in the growth and development of each of these different plant species. In *Arabidopsis thaliana*, the *PIN3/4/7* family underwent an extensive differentiation in comparison to other plant species, indicating that *AtPIN3/4/7* may have undergone functional specification in this species. The *PIN6* group could not be identified in monocotyledons, consistent with previous studies [25]. The *PIN3* and *PIN10* subfamilies are

exclusive to dicots and monocots respectively and may have evolved independently in these lineages, based on previous studies [10, 25]. Since previous studies found that magnoliaceae emerged before the divergence of monocotyledons and dicotyledons, this suggests that in monocotyledons the *PIN3* and *PIN6* families were lost, while the PIN10 family evolved independently. The *PIN* gene subfamilies in *L. chinense* most closely resemble those of *A. trichopoda* and *A. thaliana*.

Table2: Number of PIN genes in seven species

Species	Long PIN			PIN10	PIN9	Short PIN			Total
	PIN1	PIN2	PIN3/4/7			PIN5	PIN6	PIN8	
A. thaliana	1	1	3	0	0	1	1	1	8
A. trichopoda	2	1	1	0	0	1	1	3	9
O. sativa	3	1	0	2	1	0	4	1	12
S. bicolor	3	1	0	2	1	0	2	0	9
V .vinifera	2	1	0	0	0	2	2	1	8
Z. mayz	4	0	0	2	1	0	4	1	12
L. chinense	3	1	1	0	0	2	2	2	11

LcPIN protein gene structure and transmembrane topology

To better understand *L. chinense PIN* gene structure diversity and transmembrane topology, we constructed a phylogenetic tree using the *PIN* gene sequences. Gene structure patterns are highly conserved in *LcPIN* genes, with each gene containing 3-5 introns (Fig 2). The difference in gene size between the largest gene *LcPIN1a* and the smallest gene *LcPIN6a* is mainly due to intron length. Therefore, it's possible that the diversification of exons/introns played an important role in the evolution of this gene family, but the exact mechanism is unclear.

Their predicted transmembrane topology showed that, the number of hydrophobic loops in *LcPIN* proteins has a high degree of variance. Excluding *LcPIN2* and *LcPIN6b*, *LcPIN* proteins have a typical conserved structure with two highly conserved hydrophobic loops at the N and C terminus and a central hydrophilic loop within each terminus (Fig S2).

LcPIN geneshave highly conserved motifs and evolutionary relationships within different species

To further explore the evolution of the *LcPIN* gene family, we obtained 156 *PIN* amino acid sequences from Phytozome, of 17 different plant species belonging to 14 plant families: *Bryophylla* (*M. polymorpha, P. patens*), *Selaginella* (*S. moellendorffii*), *Amborellaceae* (*A. trichopoda*), *Lauraceae* (*C.kanehirae*), *Magnoliaceae* (*L. chinense*), *Gramineae* (*Z. mays, S. bicolor, O. sativa*), *Cruciferae* (*A. thaliana*), *Leguminosae* (*G.max*), *Malvaceae* (*G. raimondii*), *Vitaceae* (*V. vinifera*), *Euphorbiaceae* (*M. esculenta*), *Rosaceae* (*C. sinensis*), *Salicaceae* (*P. trichocarpa*) and *Solanaceae* (*S. lycopersicum*), and used these to constructed a neighbor-joining phylogenetic tree (Fig S2). The gene name and number of each *PIN* gene

per species is indicated in Fig S4. Most of the sequences from different species within a single subfamily clustered together to form an independent group.

We then performed motif analysis using MEME/MAST, which showed highly conserved sequences (referred to as “Motifs” numbered from 1 upwards, starting at the N-terminus) present at both the protein N- and C-termini (Fig S2). Motif1-8, 12 and 16 were found or partially found in conserved sequence regions including the two transmembrane regions. Comparisons of motif distributions revealed that the intermediately hydrophilic region of *PIN* proteins was variable across different subgroups. It could furthermore be derived that two different types of *PIN* proteins can be distinguished during *PIN* gene evolution. The first group of sequences contains a short hydrophilic loop in between two conserved transmembrane regions and can be considered a “Short PIN”. This group of *PIN* proteins is represented in *Arabidopsis* by *PIN5* and *PIN8* [30]. Our result shows that *PIN6* and *PIN9* belong to the “Short PINs” as well. A second group of *PIN* proteins contains a longer hydrophilic loop between the two transmembrane regions and can be considered a “Long PIN”. This type is represented in *Arabidopsis* by *PIN1-PIN4* and *PIN7* [30]. The *PIN1, PIN2, PIN3/4/7, PIN10* and some *PIN* genes from *Bryophylla* and *Gymnosperm* belong to the “Long PIN” in our evolutionary tree. This suggests that the “Long PIN” could have independently differentiated in Angiosperms, Gymnosperms, and Bryophytes.

As summarized in *LcPIN*, In the “Short PIN”, the *LcPIN5* only contain Motif1-8,12 and 16 withmainly distributed in the hydrophobic region at both ends of the protein. The *LcPIN8* lacked motif 1,3,7,12 in the N-terminus and there is Motif11 in the middle hydrophilic loop. In the “Long PIN”, the Motif numbers of LcPIN range is 17~20 and they have highly conservative in the hydrophilic and hydrophobic. According to the number of motifs in the middle hydrophilic area of *PIN6* was divided into two clades, Motif 9,10 of hydrophilic loop specifity appear in *LcPIN6b*. This branch number of motif was clost to “Long PIN”. *LcPIN6a* was close to the “Short PIN” for the Motifs were distributed only in hydrophobic regions. In the whole, the increase in the number of *PIN6* motifs was more like a transition from “Short PIN” to “Long PIN”. Combing with the PIN6 in the *Amborella trichopoda*, we guessed the sequences of Motif 9/10 could be believed to the symbol of the appearance of a long PIN. Compared to the “Short PINs”, the“Long PINs” possess a complex hydrophilic loop, including almost all sequence motifs save for those found in the conserved region. However, additions or deletions to individual motifs between and within subgroups could be found in the middle hydrophilic loop.

We constructed an evolutionary tree by multiple sequence alignment (Fig S2) and found a close evolutionary relationship between all *LcPINs* and *PIN* proteins from *A. trichopoda* and *C. kanehirae,* indicating that it could be a more recent evolutionary relationship to these two subgroups. A separate analysis of each subgroup reveals an interesting phenomenon: the short PINs are divided before long PINs and the *gramineae* (grasses) *PIN1/8* subgroups evolved into a separate branch before *LcPIN1/8* emerged (Fig S2). This result suggests that the long PINs could play a specific functional moving in particular direction by the deepening of differentiation and the PIN1/8 subgroups could exist the difference of funcition in monocotyledons and dicotyledons. However, this specific conclusion still needs further analysis.

Functional site analysis within *L. chinense PIN* conserved motifs

Through previous experimental verification and data analysis, several functional elements and sites that control *PIN* protein polarity, trafficking and activity have been identified [3, 31, 32]. Our multiple sequence alignments show that these elements reside for a large part within the highly conserved *LcPIN* sequence motifs (Fig S2). For example, motif1 and motif5 contain two cysteine residues (C39 and C521) and occur in the transmembrane domain of all *LcPIN* proteins, excluding only *LcPIN8* which only contains motif5 (All functional sites are labeled with *LcPIN1a* as a reference) (Fig 3 and Additional file 3). This motif has been implicated in regulating the endocytosis and distribution on the PM of “Long PINs” [33], while for “Short PINs” the functionality of these sites has not yet been verified.

The hydrophilic loop (HL) domain contains identifiable motifs as well, being motifs 4, 7, 9 and 15 (Additional file 2). Previous studies have shown that these motifs in the *PIN* protein HL contain sites involved in regulating *PIN* protein membrane abundance, as well as in maintaining their polar localization in the cell [34–37]. For example, the NPXXY element (Motif 4) near the C-terminus plays an important role in *AtPIN1* localization (Fig. 3 & Additional file 3, Mravec et al., 2009).

Motif15 contains a conserved phenylalanine residue (F165) in all “Long PIN” protein sequences of *L. chinense* (Fig 3). This residue has previously been found to interact with μA- and μD-adaptins in *vitro* and is possibly involved in *PIN1* trafficking and polar localization in *A. thaliana* [37].

Coordinated PIN-mediated auxin transport requires activation and polarity control *via* phosphorylation by protein kinases [38]. So far, the phosphorylation of *PIN* is known to be controlled by the following three protein families: AGC kinases, PROTEIN (MAP) KINASES (MPKs), and Ca2+/calmodulin-dependent protein kinase-related kinases (CRKs) [31]. We found a small number of phosphorylation sites associated with these kinases, such as S1~S3 (motif9) and T1~T3 (motif17, not all *PINs* have this motif), that are highly conserved (Additional file 3). These sites inside highly conserved TPRXS motifs and are phosphorylated by MPK4/6 kinases [32], suggesting possible coregulation. Part of the sites, such as T1/T2 (motif9) were different in individual *PIN* proteins of different species. For example, Thr is replaced by Gln in some gramineous plants. The protein kinases PID/WAGs and D6PK directly phosphorylate *PINs* mainly through three conserved serine sites: S1, S2 and S3, activating *PIN*-mediated auxin efflux and regulating *PIN* polarity (Barbosa et al., 2018; Hammouti et al., 2016; Huang et al.,2010).

Our multiple sequence alignment showed that these sites of significance are highly conserved in the motifs found in the “Long PIN” HL domain and all *PIN* transmembrane domains. We could only detect sequence divergence in individual angiosperm sequences. These results suggest that the conserved sites in multiple motifs may have a fundamental function in different species.

Organ-specific expression profile of the *LcPIN* family genes

We used fluorescence quantitative PCR to analyze the expression of *LcPIN* gene family members in different organs (stamen, pistil, petal, bark, bud, root, stem, leaf) of *L. chinense*. Since the *LcPIN5a-1/5a-2*

and *LcPIN8a-1/8a-2* sequences are identical, it was difficult to design specific expression primers to distinguish them. Therefore, the expression level of these genes is the combined expression of two gene copies. We found that the *LcPIN* gene family members are expressed in almost all *L. chinense* tissues (Fig 4). However, the relative expression levels of *LcPIN8a-1/8a-2* and *LcPIN1b* are comparatively low and absent from some tissues. *LcPIN3* and *LcPIN6a* are highly expressed in all tissues and *LcPIN1c* is expressed in multiple tissues except the petal and leaf. The expression patterns of *LcPIN1a* and *LcPIN5* show high similarity, with higher expression in the bud and leaf than in other tissues. *LcPIN2* and *LcPIN6b* showed more specific expression patterns in a more limited number of tissues, with *LcPIN2* mainly expressed in the root and stamen and *LcPIN6b* mainly in buds. Interestingly, we found that *LcPIN3* and *LcPIN6a* show relatively high expression in stamens and petals. The upper part of the *L. chinense* petal is covered with green, and the color of middle and lower part becomes weak compared with the *L. tulipifera*. To further observe the changes of PIN genes in stamens and petals. We established the dynamic expression pattern in different periods of petals and stamens. In the stamens, *LcPIN6a*, *LcPIN3*, *LcPIN1a* and *LcPIN1c* was expressed (Additional file 4). The *LcPIN3* had a higher level of expression than LcPIN1a/1c (Fig 5A). At the same time, its level of expression has been declining from LC-1 to LC-3 and increased in LC-4. Comparing with the *LcPIN3*, the changed of *LcPIN1a* and *LcPIN1c* was more stabilization (Fig 5A &Additional file 5). The *LcPIN3* and *LcPIN6a* displayed a similar expression profiles in the same part of petals and *LcPIN1a* had a low expression except in the upper part of LC-4 stage of petal development (Fig 5A & Additional file 5). On the lower middle part of the petal in the LC-4 stage, the *LcPIN3* and *LcPIN6a* expression levels rose sharply (Fig 5C &5D). Before this stage, their expression level was relatively stable in the middle of the petal. To summarize the above, the *LcPIN3* could display certain influence on the development of stamens and the *LcPIN3* and *LcPIN6a* play a role in the development of petal.

PIN genes dynamic expression patterns during the somatic embryogenesis in *Liriodendron × sinoamericanum*（154102）

As an auxin efflux protein, *PIN* plays an important role during the growth and development of many plant species [39]. Though the *Liriodendron × sinoamericanum* artificial hybrid (obtained from the Chinese and North American *Liriodendron* subspecies) acquired immature embryos induced embryogenic callus. Based on previously generated RNA-Seq data that was obtained from successive developmental stages during somatic embryogenesis, we constructed a heat-map showing the expression of all 11 *LcPINs* at each stage (Globular embryo, Heart-shaped embryo, Torpedo embryo, Immature cotyledon embryo, Cotyledon embryo and Plantlet). Barely detectable or no expression was observed for *LcPIN5a-1/5a-2, LcPIN8a-1/8a-2* and *LcPIN6a/6b* (Fig 6), which indicates that they might not express during embryogenesis or are only activated under special conditions. The other *LcPIN* (Long PIN) gene expression patterns were divided into two groups. *LcPIN2* and *LcPIN1b* expression was barely detectable at the globular embryo stage, after which expression levels rose until the immature cotyledon embryo stage. The *LcPIN1a*, *LcPIN1c* and *LcPIN3* expression levels remained relatively constant throughout this process except at the plantlet stage. These findings suggested that the long *LcPINs* genes could play a

key role at different stages of somatic embryogenesis, while Short PINs are barely expressed and less likely to be involved.

Discussion

Basic information and evolutionary relationship of *LcPIN* gene family

In this study, we demonstrated the presence of 11 *PIN* genes in *L. chinense*, located on 6 separate chromosomes (Fig 2A). We carried out phylogenetic analyses, comparing *LcPIN* sequences to *PINs* of other plant species. The *LcPIN1/5/6/8* subgroups all possess more than one sequence, with *LcPIN5* and *LcPIN8* having a duplicate gene with up to 100% sequence identity. A previous study found that the *L. chinense* genome may have undergone whole genome duplication around 116 million years ago, an event that could underlie the presence of duplicated *PIN* genes [29]. *Liriodendron* belongs to the *magnoliaceae* family, which is thought to have diverged at the base of the angiosperm lineage [40]. Evolutionary analysis shows that the *LcPIN* and *AtrPIN* likely had a common ancestor (Fig 1). In order to better understand the diversity and similarity of the *LcPIN* genes, we analyzed their intron-exon structure (Fig 2B & Additional file 1), finding little variation overall. The main reason for gene length variation is the variation in intron length, which was also found for other species [25].

The structure domain and membership division of *LcPIN* protein

PIN genes have been studied in detail in *A. thaliana*: they are mainly composed of two conserved hydrophobic loops in the N- and C- terminus and a variable hydrophobic loop in the middle [31]. By analyzing the transmembrane domain and conserved motifs in *LcPIN* proteins, we found that most of the transmembrane structures of *LcPINs* contain 5-9 transmembrane spirals except *LcPIN6b*. But like *LcPIN2* and *LcPIN6b*, they only have transmembrane helices at the N-terminus, not at the C-terminus. However, the same conserved motif exists at the C-terminus of these proteins as it does in other species (Additional file 2). Through multi-species protein sequence comparison, it can be found that all *LcPIN* proteins have the same motif (except for *LcPIN8a/b*, which has only one motif at the N-terminus), and they jointly constitute a highly conserved domain. The sequence length of the hydrophobic loops determines whether a *PIN* gene is a “Long *PIN”* or a “Short *PIN”*. However, the hydrophobic loops were found to contain four instances of highly conserved sequence (HC1-HC4) [41]. A more accurate classification for PIN genes was made based on the degree of HC1-HC4 sequence conservation, being: “Canonical PIN”, “Noncanonical PIN”. and “Semicanonical PIN” with *PIN6* [31, 41]. This changed classification is directly related to the presence or absence of certain motifs in the proteins. Analysis of *PIN* genes family in 17 species, the most PIN genes all had conservative motif in the N and C terminal of the sequences. The difference mainly due to the diversity of the sequences in the transmembrane conservative structure and decision. The hydrophobic loop is distributed at both ends of the protein sequences include motif 1,2,3,4,5,6,7,8,12,16. And the middle of the protein sequence contains hydrophilic loop. “Canonical PIN” hydrophilic loop in the middle there were a lot of conservative motif, Therefore the distinction between different subgroups were mainly concentrated in the hydrophilic loop. Looking at *PIN6*, it can been found

that it gathers into two branches, which favor Canonical *PIN* and Noncanonical *PIN* respectively and can been regarded as a transition between these two kinds of *PIN*. This phenomenon was well represented in *LcPIN6* genes.

LcPIN has some conservative functional sites

As an auxin efflux protein, *PIN* plays an important role in transporting intercellular auxin. *PIN* proteins contain a number of functional sites located in conserved regions of the protein. However, non-conserved regions may also contain functional sites, which are often species-specific and related to species differences and sequence evolution [4, 9, 11, 16, 18, 30–32, 34, 35]. Here, we limit ourselves to the discussion of conserved functional sites concerning auxin transport, protein polarity localization and protein activity (Additional file 2 & Additional file 3 & Fig. 3). The conserved C39/C521/F165 sites in *LcPINs* may participate in the interaction with several μ-adaptins to control trafficking and polarity localization on the plasma membrane in Long PINs. F165 mutation resulted in accumulation of the carrier in round, condensed structures in *AtPIN1* [37].

The three predicted TPR*S (motif9) sites are binding sites for different phosphatases that activate *PIN* protein, but in *LcPIN1c*, there were only two TPR*S regions (lacking T3-S3), indicating some diversity among *LcPIN* proteins. The di-acidic motif is composed of the tyrosine motif NPNXY followed by an SSL sequence. Alignment of the region spanning the tyrosine motif of all *LcPIN* proteins revealed a conserved sequence NPN(S/T) -YSSL (where S is found in the *LcPIN5/6a* sequence and T is present in other *LcPIN*s) in the HL of *LcPIN*s, with the last three SSL amino acids missing in the short-looped *LcPIN5/6/8*. Mutagenesis of the di-acidic motif in *AtPIN1* resulted in significant accumulation of the protein at the endoplasmic reticulum [21], supporting its role in trafficking from the endoplasmic reticulum. It suggests that this motif may have a similar function in *L. chinense.*

The expression pattern of LcPIN gene was preliminarily revealed

While studying the structure of *PIN* proteins, corresponding functional studies have also been carried out. As an inter-cellular auxin output protein, *PIN* plays an essential role in the growth and development of plants [4, 9, 12, 20, 21, 26, 40]. There are multiple members in most subgroups of the *LcPIN* gene family. Each subgroup has its own pattern of expression. *LcPIN1/6* subgroup expression was found across eight different tissues, but each member of this subgroup expressed in a different subset of these tissues. For example, the *LcPIN1a* were expression in eight tissue but the *LcPIN1b* almost no expression in flower. Similarly, *LcPIN3* is expressed in all these same tissues, but its expression level in stamens is several times higher than in other tissues. At*PIN2* was also observed in the region where root hairs are formed [42], but *LcPIN2* was highly expressed in both the root and stamen. This suggests that *LcPIN2* could have evolved additional areas of expression. We found that *LcPIN3* shows high and dynamic expression levels in petals and stamens. The *PIN6* expression in the nectary influences nectar enrichment in *Arabidopsis thaliana* [47], with *LcPIN6a* being expressed in the lower middle part of petals it could exert a similar function due to the presence of nectary tissue in this region [40].

Somatic embryogenesis is a special phenomenon that can occur in plants and which shows many parallels to zygotic embryo development. Therefore, somatic embryogenesis can be used as a model to further understand the process of zygotic embryogenesis. Combined with transcriptome sequencing, we preliminarily revealed the expression pattern of the *LcPIN* gene family during somatic embryogenesis of *Liriodendron × sinoamericanum.* This process is mainly due to the participation of “Canonical PIN”. During the embryo development of *Arabidopsis thaliana*, auxin was excreted mainly by *AtPIN1/3/4/7* [43–45]. This process is similar to *Liriodendron × sinoamericanum*. However, the expression of *LcPIN* members varied at different stages. It is suggested that it's not a single *LcPIN* gene involved in this process. There is a close evolutionary relationship between the *PIN3/4/7* in the evolutionarily. The *LcPIN3* could have the function of *PIN3/4/7* in the Somatic embryogenesis with the evolutionary status of magnoliaceae. Interestingly, *LcPIN2* is also expressed in this process, indicating that *LcPIN2* may have some special functions.

Conclusion

Through the above analysis, we analyzed the *LcPIN* gene family from gene structure, protein properties, transmembrane domains, evolutionary relationships, etc. The gene expression pattern was preliminarily explored in different tissues and Somatic embryogenesis. The specific expression of LcPIN3 and LcPIN6a in flowers was expression expecially, which provided the basis for the verification of gene function and the breeding of leydena.

Methods

Identification of *PIN* Genes in the *L. chinense* genome

L. chinense protein sequences were downloaded from (https://hardwoodgenomics.org/genomes/page=2). *Arabidopsis PIN* protein sequences were acquired from TAIR1.0. We used HMMER3.0 software and the pfam number of *PIN* (PF03547) to search for *LcPIN* protein sequences. The corresponding sequences were identified based on the BLAST program using the *AtPIN1-8* protein sequences as queries. Next, the *LcPIN* sequences were further authenticated based on the conserved domains using SMART (http://smart.emblheidelberg.de). Biochemical properties, such as the molecular weight (kDa) and isoelectric point (pI) of each protein, were determined using the Compute pI/Mw tool on the ExPASy (https://web.expasy.org/protparam/) website (Table1). The locations of the *LcPIN* genes on each chromosome were determined based on the *L. Chinense* genome sequence.

Multiple sequence alignment and phylogenetic analysis of *L. chinense PIN* gene family

The *PIN* protein sequences of six species (*Amborella trichopoda, Arabidopsis thaliana, Zea mays, Oryza sativa, Vitis vinifera and Sorghum bicolor*) were downloaded from Phytozome (Additional file 4). The phylogenetic relationships between *LcPINs* and the *PIN* proteins from these six species were determined

using the neighbor-joining algorithm set to default parameters with 1000 bootstrap analyses, using MEGA 7.0 software (Fig 1). The *LcPINs* were named according to their respective clades.

Gene structure, chromosomal localization and transmembrane topology analysis of *LcPINs*

The chromosomal locations of the *LcPIN* genes were determined based on the *L. chinense* genome (Fig 2A). Their exon/intron structure was determined with Tbtools using mRNA and the *L. chinense* genome. The genomic and coding sequences of PIN genes, together with their exon/intron structures, were extracted from the general feature format (GFF3) file of *L. chinense* sequences (Fig 2B). Protein transmembrane topology was predicted using the TMHHM server 2.0 (http://www.cbs.dtu.dk/services/TMHMM) (Additional file 1).

Multiple sequence alignment and identification of conserved motif in *PIN* gene family of 17 plant species

We retrieved the *A. thaliana PIN* protein sequences from the Arabidopsis Information Resource database (www.arabidopsis.org). A BLASTP search was performed using the *AtPIN* sequences as query to retrieve *PIN* sequences from the Phytozome database. Sequences were retrieved from the plant species *M. polymorpha, P. patens, S. moellendorffii, A. trichopoda, C. kanehirae, Z. mays, S. bicolor, O. sativa, A. thaliana, G. max, G. raimondii, V. vinifera, M. esculenta, C. sinensis, P. trichocarpa* and *S. lycopersicum* (Additional file 4). Sequence alignment was performed using MEGA 7.0. Sequence relationships were inferred using the NJ method. Conserved motifs were checked using the online Multiple Expectation Maximization for Motif Elicitation (MEME) program. The repetition was set as any number with an optimal width of 6–100 residues and the maximum number of motifs as 20.

L. chinense PIN gene family expression analysis and dynamic expression patterns during somatic embryogenesis in *Liriodendron × sinoamericanum*（Genotype:154102）

Samples of eight organs (stames, pistil, petal, bark, bud, root, stems, leaf) were taken from the baima test site at Nanjing Forestry university. Total RNA extraction was performed using a chloroform-free plant total RNA extraction kit from BioTeke corporation. qRT-PCR analysis was conducted following standard procedure and using three biological replicates [46]. All primers for qRT-PCR were designed by Primer5.0 and are listed in Table3.

For expression analysis during somatic embryogenesis of *Liriodendron × sinoamericanum*, materials at different developmental stages (Globular embryo, Heart-shaped embryo, Torpedo embryo, Immature cotyledon embryo, Cotyledon embryo and Plantlet) were collected and transcriptome sequencing was performed. RNA-seq data has not yet been released. Using FPKM data, we constructed expression profiles.

Table3: qRT-PCR primers used for LcPIN genes

Gene Name		qRT-PCR Primers
LcPIN 1a	F	AAGAGCCGCAAGAAGAGT
	R	TGTTTGGATTACGGATTAGC
LcPIN 1b	F	AACAAGTCCAGGTTCCATT
	R	ATCTTTCGTCCCTTCGTC
LcPIN 1c	F	GGGAGGAACACGAGCAAT
	R	CTTCTTCTTCGCCACAGC
LcPIN 2	F	CAGAAGAAGGCTGACGT
	R	AATGAGGCTGGAATAGGTG
LcPIN 3	F	GAGAAGAGGGATGGGAGAAG
	R	ATGACACTGGCAGGAGGC
LcPIN 5a-1	F	AGACAACCAGCGACATCA
	R	TCGCGTAAGAGTTAGGATTA
LcPIN 5a-2	F	AGACAACCAGCGACATCA
	R	TCGCGTAAGAGTTAGGATTA
LcPIN 6a	F	GCAGAGTGATAGCAAGGTG
	R	CGACGACACTGACGGATT
LcPIN 6b	F	TCATGGTATCGCTGACGT
	R	GACCCAGATCGGCGTATT
LcPIN 8a-1	F	GGAGAATCAAGGAGAAAC
	R	CAAATGAGACCAACCAAA
LcPIN 8a-2	F	GGAGAATCAAGGAGAAAC
	R	CAAATGAGACCAACCAAA

Abbreviations

PIN: PIN-FORMED;

CDS: Coding sequence;

FPKM: Fragments per kilobase of exon per million fragments mapped;

ORF: Open reading frame;

UTR: Un-translated regions;

qRT-PCR: Quantitative real-time PCR;

IAA: Indole-3-acetic acid.

Declarations

1.Ethics approval and consent to participate

Not applicable

2.Consent for publication

Not applicable

3.Availability of data and materials

The data sets supporting the results of this article are included within the article and its additional files.

4.Competing interests

The authors declare that they have no competing interests

5.Funding

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6.Authors' contributions

LFH, JSS, JHC, PKW and TLC contributed to the management and manuscript review. PKW designed experiments as well as provided the methodology of data collection and analysis. WHW, JJZ and LFH helped with the collection of samples. LFH performed the qRT-PCR experiment and analyed of PIN evolution. All authors have read and approved the final manuscript

7. Acknowledgements

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References

1. Paponov IA, Teale WD, Trebar M, Blilou I, Palme K. The PIN auxin efflux facilitators: Evolutionary and functional perspectives. Trends Plant Sci. 2005;10:170–7.
2. Friml J. Auxin transport Ð shaping the plant Prelude Ð The linden tree of innocence. Curr Opin Plant Biol. 2003;6:7–12. doi:10.1016/S1369-5266(02)00003-1.
3. Heisler MG, Ohno C, Das P, Sieber P, Reddy G V., Long JA, et al. Patterns of auxin transport and gene expression during primordium development revealed by live imaging of the Arabidopsis inflorescence

meristem. Curr Biol. 2005;15:1899–911.

4. Reinhardt D, Mandel T, Kuhlemeier C. Auxin regulates the initiation and radial position of plant lateral organs. Plant Cell. 2000;12:507–18.
5. Reinhardt D, Pesce ER, Stieger P, Mandel T, Baltensperger K, Bennett M, et al. Regulation of phyllotaxis by polar auxin transport. Nature. 2003;426:255–60.
6. Remy E, Cabrito TR, Baster P, Batista RA, Teixeira MC, Friml J, et al. A Major Facilitator Superfamily transporter plays a dual role in polar auxin transport and drought stress tolerance in Arabidopsis. Plant Cell. 2013;25:901–26.
7. Tanaka H, Dhonukshe P, Brewer PB, Friml J. Spatiotemporal asymmetric auxin distribution: A means to coordinate plant development. Cell Mol Life Sci. 2006;63:2738–54.
8. Wang HZ, Yang KZ, Zou JJ, Zhu LL, Xie ZD, Morita MT, et al. Transcriptional regulation of PIN genes by FOUR LIPS and MYB88 during Arabidopsis root gravitropism. Nat Commun. 2015;6 June.
9. Zhao Y. Auxin Biosynthesis and Its Role in Plant Development. Annu Rev Plant Biol. 2010;61:49–64.
10. Adamowski M. PIN-Dependent Auxin Transport : Action , Regulation , and Evolution. 2015;27 January:20–32.
11. Benková E, Michniewicz M, Sauer M, Teichmann T, Seifertová D, Jürgens G, et al. Local, Efflux-Dependent Auxin Gradients as a Common Module for Plant Organ Formation. Cell. 2003;115:591–602.
12. Rowe JH, Topping JF, Liu J, Lindsey K. Abscisic acid regulates root growth under osmotic stress conditions via an interacting hormonal network with cytokinin, ethylene and auxin. New Phytol. 2016;211:225–39.
13. Péret B, Swarup K, Ferguson A, Seth M, Yang Y, Dhondt S, et al. AUX/LAX genes encode a family of auxin influx transporters that perform distinct functions during arabidopsis development. Plant Cell. 2012;24:2874–85.
14. Sanders HL, Langdale JA. Conserved transport mechanisms but distinct auxin responses govern shoot patterning in Selaginella kraussiana. New Phytol. 2013;198:419–28.
15. Gälweiler L, Guan C, Müller A, Wisman E, Mendgen K, Yephremov A, et al. Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue. Science (80-). 1998;282:2226–30.
16. Benjamins R, Scheres B. Auxin: The Looping Star in Plant Development. Annu Rev Plant Biol. 2008;59:443–65.
17. Adamowski M, Friml J. PIN-dependent auxin transport: Action, regulation, and evolution. Plant Cell. 2015;27:20–32.
18. Barbez E, Kubeš M, Rolčík J, Béziat C, Pěnčík A, Wang B, et al. A novel putative auxin carrier family regulates intracellular auxin homeostasis in plants. Nature. 2012;485:119–22.
19. Cazzonelli CI, Vanstraelen M, Simon S, Yin K, Carron-Arthur A, Nisar N, et al. Role of the Arabidopsis PIN6 Auxin Transporter in Auxin Homeostasis and Auxin-Mediated Development. PLoS One. 2013;8.

20. Ding Z, Wang B, Moreno I, Duplá Ková N, Simon S, Carraro N, et al. ER-localized auxin transporter PIN8 regulates auxin homeostasis and male gametophyte development in Arabidopsis. Nat Commun. 2012;3 July.
21. Mravec J, Skůpa P, Bailly A, Hoyerová K, Křeček P, Bielach A, et al. Subcellular homeostasis of phytohormone auxin is mediated by the ER-localized PIN5 transporter. Nature. 2009;459:1136–40.
22. Křeček P, Skůpa P, Libus J, Naramoto S, Tejos R, Friml J, et al. The PIN-FORMED (PIN) protein family of auxin transporters. Genome Biol. 2009;10:1–11.
23. Dhonukshe P, Huang F, Galvan-Ampudia CS, Ma¨ho¨nen AP, Kleinevehn J, Xu J, et al. Plasma membrane-bound AGC3 kinases phosphorylate PIN auxin carriers at TPRXS(N/S) motifs to direct apical PIN recycling. Dev. 2015;142:2386–7.
24. Wang JR, Hu H, Wang GH, Li J, Chen JY, Wu P. Expression of PIN genes in rice (Oryza sativa L.): Tissue specificity and regulation by hormones. Mol Plant. 2009;2:823–31. doi:10.1093/mp/ssp023.
25. Wang Y, Chai C, Valliyodan B, Maupin C, Annen B, Nguyen HT. Genome-wide analysis and expression profiling of the PIN auxin transporter gene family in soybean (Glycine max). BMC Genomics. 2015;16:1–13.
26. Zhang Y, He P, Yang Z, Huang G, Wang L, Pang C, et al. A genome-scale analysis of the PIN gene family reveals its functions in cotton fiber development. Front Plant Sci. 2017;8 March:1–13.
27. Forestan C, Farinati S, Varotto S. The maize PIN gene family of auxin transporters. Front Plant Sci. 2012;3 FEB:1–23.
28. Feraru E, Vosolsobě S, Feraru MI, Petrášek J, Kleine-Vehn J. Evolution and structural diversification of PILS putative auxin carriers in plants. Front Plant Sci. 2012;3 OCT:1–13.
29. Chen J, Hao Z, Guang X, Zhao C, Wang P, Xue L, et al. Liriodendron genome sheds light on angiosperm phylogeny and species–pair differentiation. Nat Plants. 2019;5:18–25.
30. Viaene T, Delwiche CF, Rensing SA, Friml J. Origin and evolution of PIN auxin transporters in the green lineage. Trends Plant Sci. 2013;18:5–10.
31. Zwiewka M, Bilanovičová V, Seifu YW, Nodzyński T. The Nuts and Bolts of PIN Auxin Efflux Carriers. Front Plant Sci. 2019;10 July.
32. Barbosa ICR, Hammes UZ, Schwechheimer C. Activation and Polarity Control of PIN-FORMED Auxin Transporters by Phosphorylation. Trends Plant Sci. 2018;23:523–38.
33. Retzer K, Lacek J, Skokan R, Genio CI, Konstantinova N. Evolutionary Conserved Cysteines Function as cis -Acting Regulators of Arabidopsis PIN-FORMED 2 Distribution. 2017.
34. Barbosa ICR, Schwechheimer C. Dynamic control of auxin transport-dependent growth by AGCVIII protein kinases. Curr Opin Plant Biol. 2014;22:108.
35. Weller B, Zourelidou M, Frank L, Barbosa ICR, Fastner A, Richter S, et al. Dynamic PIN-FORMED auxin efflux carrier phosphorylation at the plasma membrane controls auxin efflux-dependent growth. Proc Natl Acad Sci U S A. 2017;114:E887–96.

36. Traub LM. Tickets to ride: Selecting cargo for clathrin-regulated internalization. Nat Rev Mol Cell Biol. 2009;10:583–96.
37. Sancho-Andrés G, Soriano-Ortega E, Gao C, Bernabé-Orts JM, Narasimhan M, Müller AO, et al. Sorting motifs involved in the trafficking and localization of the PIN1 auxin efflux carrier. Plant Physiol. 2016;171:1965–82.
38. Lee SH, Cho HT. Erratum: PINOID positively regulates auxin efflux in Arabidopsis root hair cells and tobacco cells (The Plant Cell (2006) 18 (1604-1616)). Plant Cell. 2006;18:2094.
39. He P, Zhao P, Wang L, Zhang Y, Wang X, Xiao H, et al. The PIN gene family in cotton (Gossypium hirsutum): genome-wide identification and gene expression analyses during root development and abiotic stress responses. 2017;:1–10.
40. Liu H, Ma J, Li H. Transcriptomic and microstructural analyses in Liriodendron tulipifera Linn. Reveal candidate genes involved in nectary development and nectar secretion. BMC Plant Biol. 2019;19:1–18.
41. Bennett T, Brockington SF, Rothfels C, Graham SW, Stevenson D, Kutchan T, et al. Paralogous Radiations of PIN Proteins with Multiple Origins of Noncanonical PIN Structure. 2014;31:2042–60.
42. Friml J, Yang X, Michniewicz M, Weijers D, Quint A, Tietz O, et al. A PINOID-dependent binary switch in apical-basal PIN polar targeting directs auxin efflux. Science (80-). 2004;306:862–5.
43. Billou I, Xu J, Wildwater M, Willemsen V, Paponov I, Frimi J, et al. The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots. Nature. 2005;433:39–44.
44. Friml J, Vieten A, Sauer M, Weijers D, Schwarz H, Hamann T, et al. Efflux-dependent auxin gradients establish the apical-basal axis of Arabidopsis. Nature. 2003;426:147–53.
45. Friml J, Benková E, Blilou I, Wisniewska J, Hamann T, Ljung K, et al. AtPIN4 mediates sink-driven auxin gradients and root patterning in Arabidopsis. Cell. 2002;108:661–73.
46. Chai C, Wang Y, Joshi T, Valliyodan B, Prince S, Michel L, et al. Soybean transcription factor ORFeome associated with drought resistance: A valuable resource to accelerate research on abiotic stress resistance. BMC Genomics. 2015;16:1–9. doi:10.1186/s12864-015-1743-6.
47. Bender RL, Fekete ML, Klinkenberg PM, Hampton M, Bauer B, Malecha M, et al. PIN6 is required for nectary auxin response and short stamen development. Plant J. 2013;74:893–904.

Supplementary Information

Additional file 1: Transmembrane topology analysis of LcPIN proteins. The protein transmembrane topology was predicted using the TMHHM server 2.0 http://www.cbs.dtu.dk/services/TMHMM/)

Additional file 2: Schematic representations of conserved motifs in PIN proteins. A phylogenetic tree of *PIN* genes from 17 plant species is indicated on the left, while conserved motifs in PIN proteins are shown on the right. Different motifs are differently colored boxes with numbers 1 to 20.

Additional file 3: Functional sites predicted in motifs of PIN gene family in 17 species. The red letters represent variable loci in this species. The asterisk "*" represents that the amino acid is diversified in this position.

Additional file 4: Evolutionary analysis of sequence basic information.

Additional file 5: Dynamic changes in the expression of *LcPIN* genes during the development of petals and stamens. LC-number: different stage; U: Upper part of the petal; M: Middle part of the petal; D: Base of the petal. The relative expression level was determined using *LcPIN1a* in the LC-1 stage of stamens as a control.

Figures

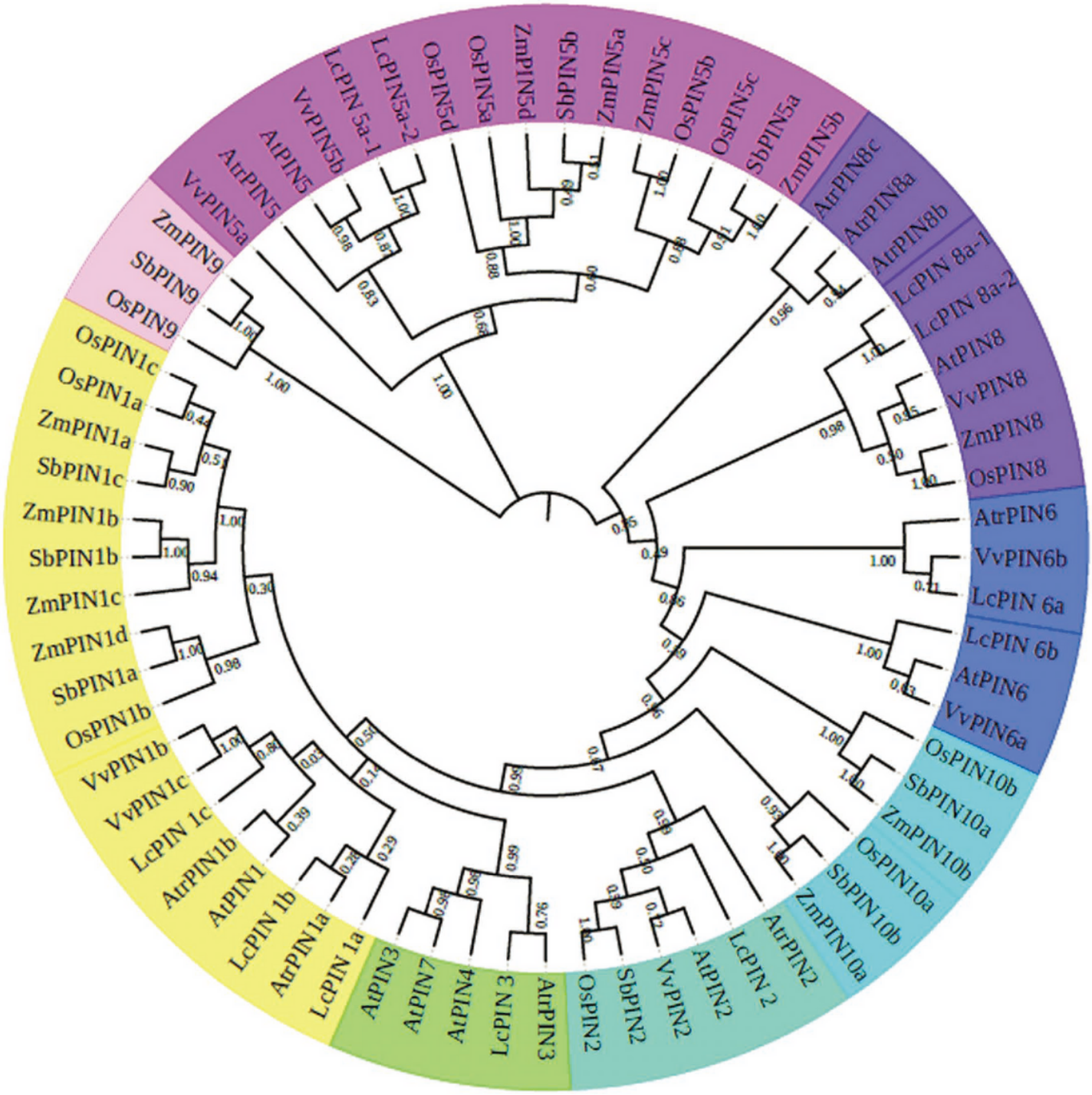

Figure 1

Phylogenetic relationships of the PIN auxin transporters protein from Liriodendron chinense (Lc), Amborella trichopoda(Atr), Arabidopsis thaliana(At), Zea mays(Zm), Oryza sativa(Os). Vitis vinifera(Vv), Sorghum bicolor(Sb). The phylogenetic tree was constructed using Mega 7.0 using the neighbor-joining (NJ) method. Different PIN subfamilies are represented by different colors.

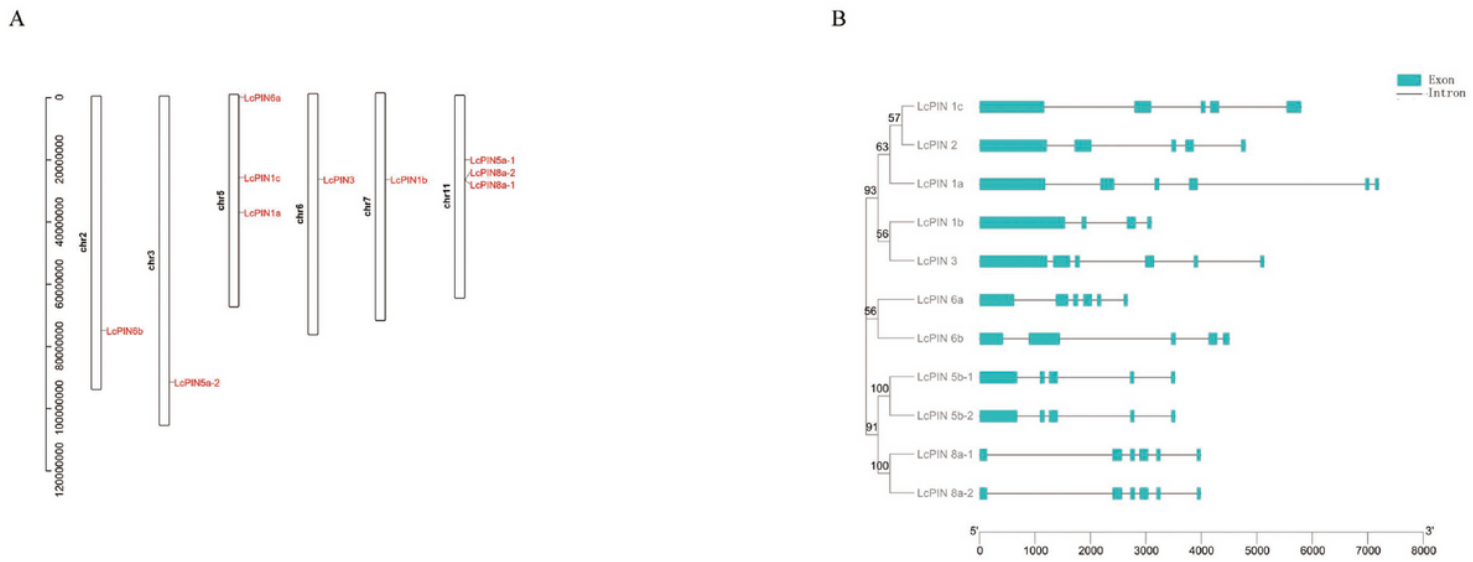

Figure 2

A: Chromosomal distributions of the identified PIN genes in L. chinense. Chromosomal locations are shown from top to bottom on corresponding chromosomes according to genome annotation. B: Gene structure and Phylogeny of 11 PIN proteins in L. chinense. The phylogenetic tree (left panel) was constructed in MEGA 7.0 using the neighbor-joining (NJ) method with 1000 bootstrap replicates. For the gene structures (right panel), exons and introns are marked with blue boxes and black lines, respectively. A scale bar indicating bp sequence length is shown at the bottom.

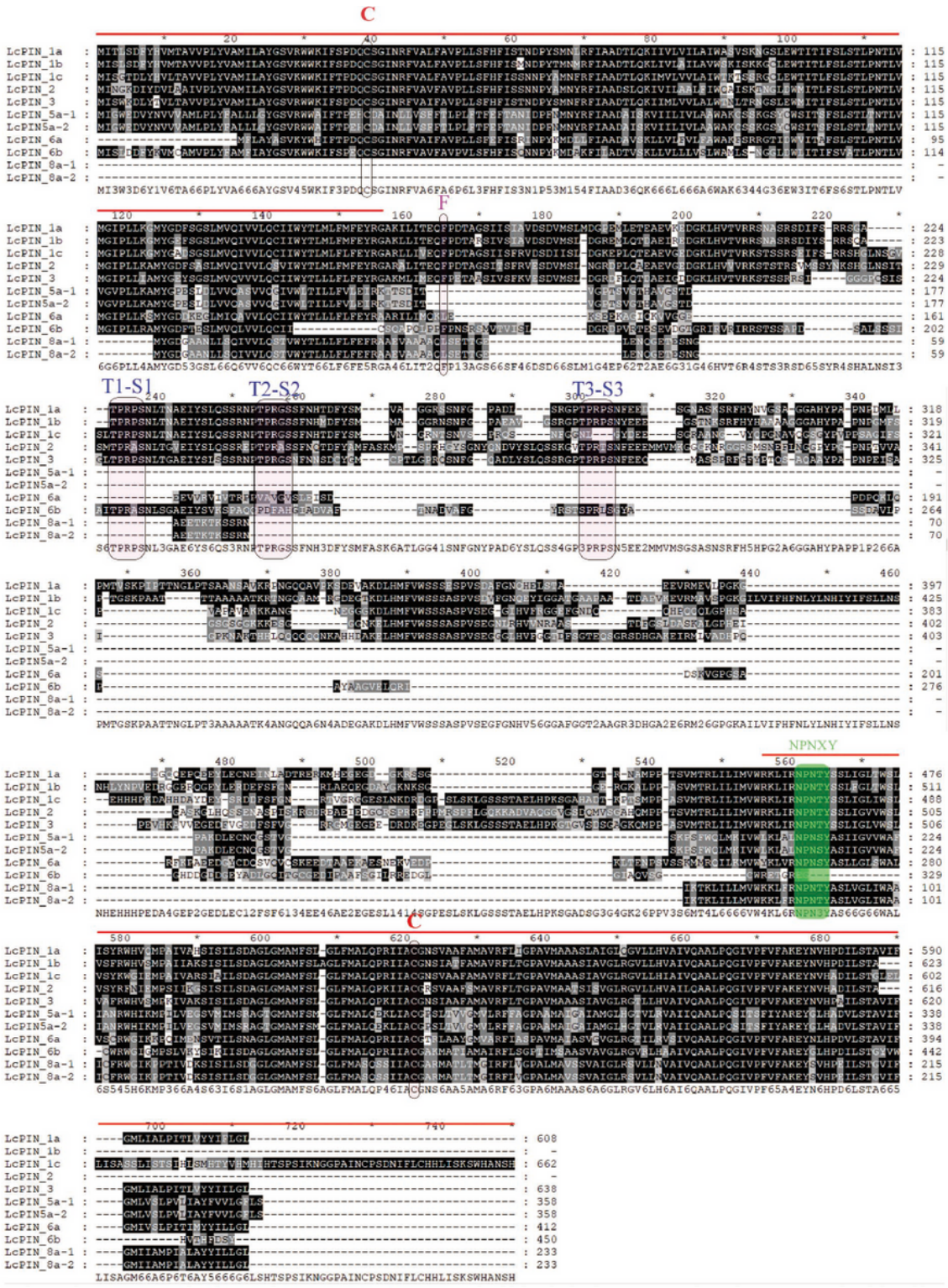

Figure 3

LcPIN genes amino acid sequence multiple alignment. The red line indicates a conserved transmembrane domain. The possible functional sites or elements are encircled by a box. Letters with different colors represent different function.

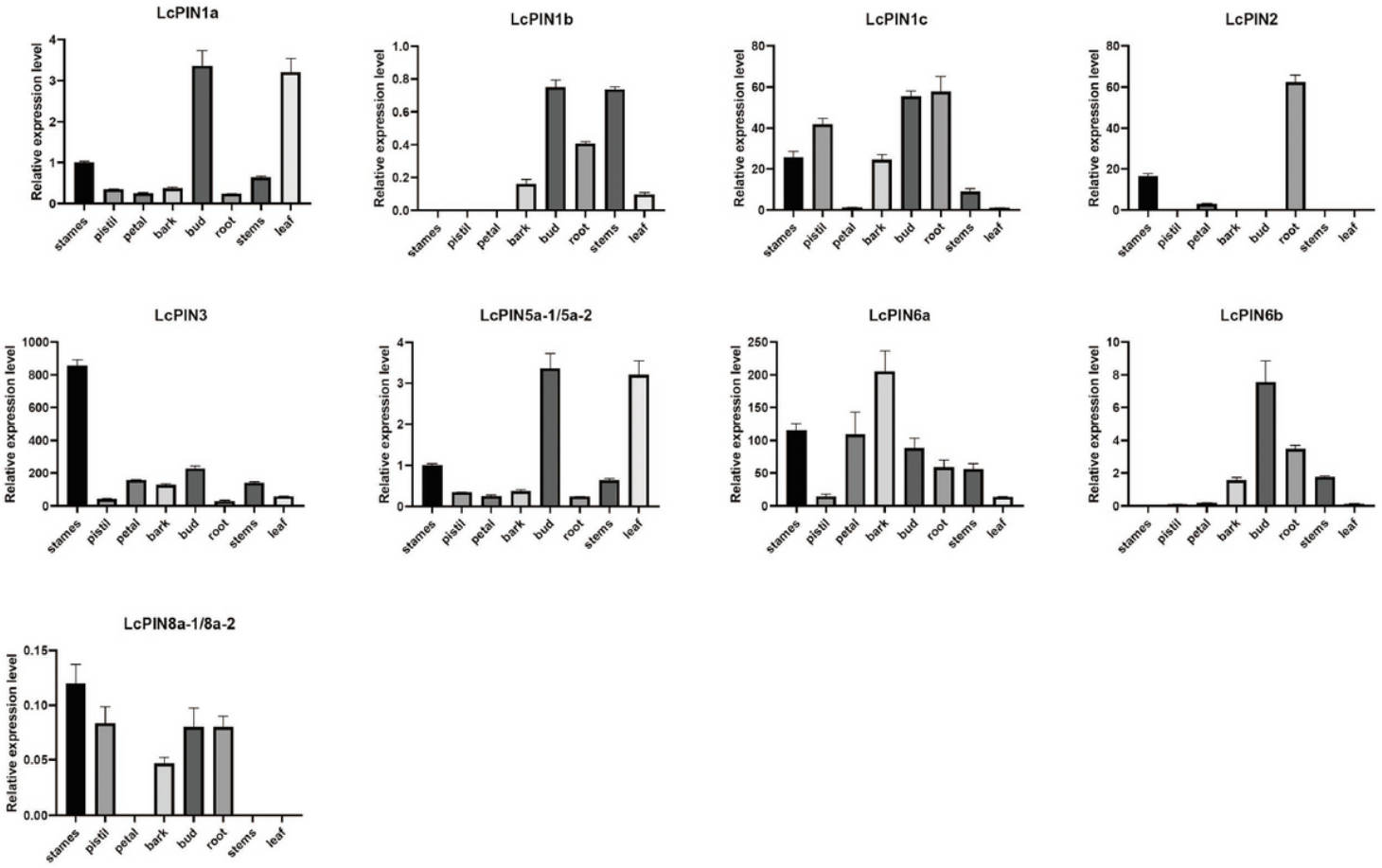

Figure 4

The relative expression of PIN genes in different L. chinense tissues. qRT-PCR experiments were performed on three biological replicates. Error bars represent means ± SE from three independent experiments. The relative expression level was determined using the expression of LcPIN1a in stamens as a control. Notice that the y-axis has been rescaled across individual graphs for visual representation.

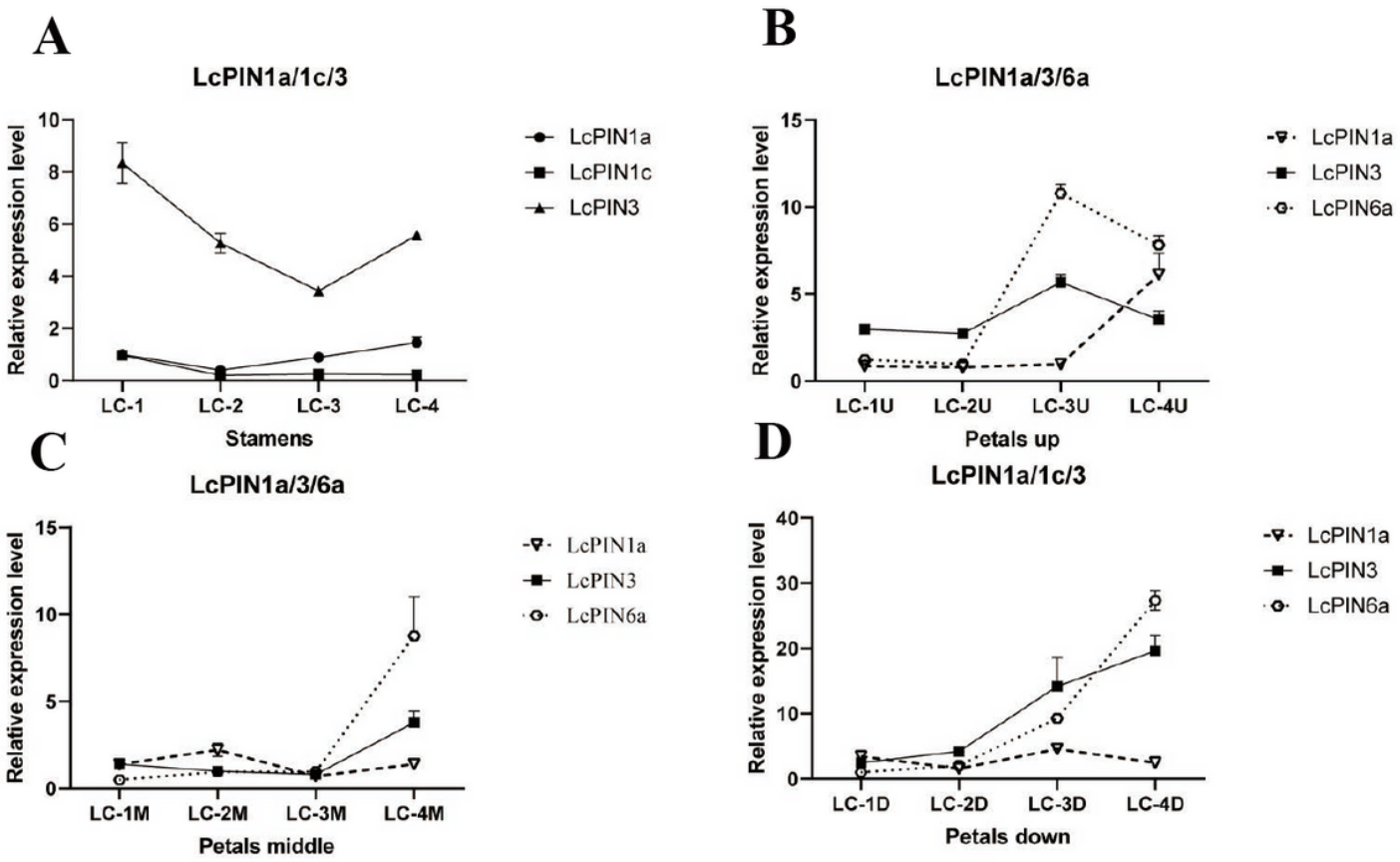

Figure 5

Expression profiling of different LcPIN genes involved in the development of stamens and petals in L. chinense. A: The expression changes of LcPIN1a/1c/3 during stamen development. B-D: The expression pattern of LcPIN1a/3/6a at different petal developmental stages and sections. LC-number: successive developmental stages; U: Upper part of the petal; M: Middle part of the petal; D: Base of the petal. Error bars represent means ± SE from three independent experiments. The relative expression level was determined using LcPIN1a in the LC-1 stage of stamens as a control.

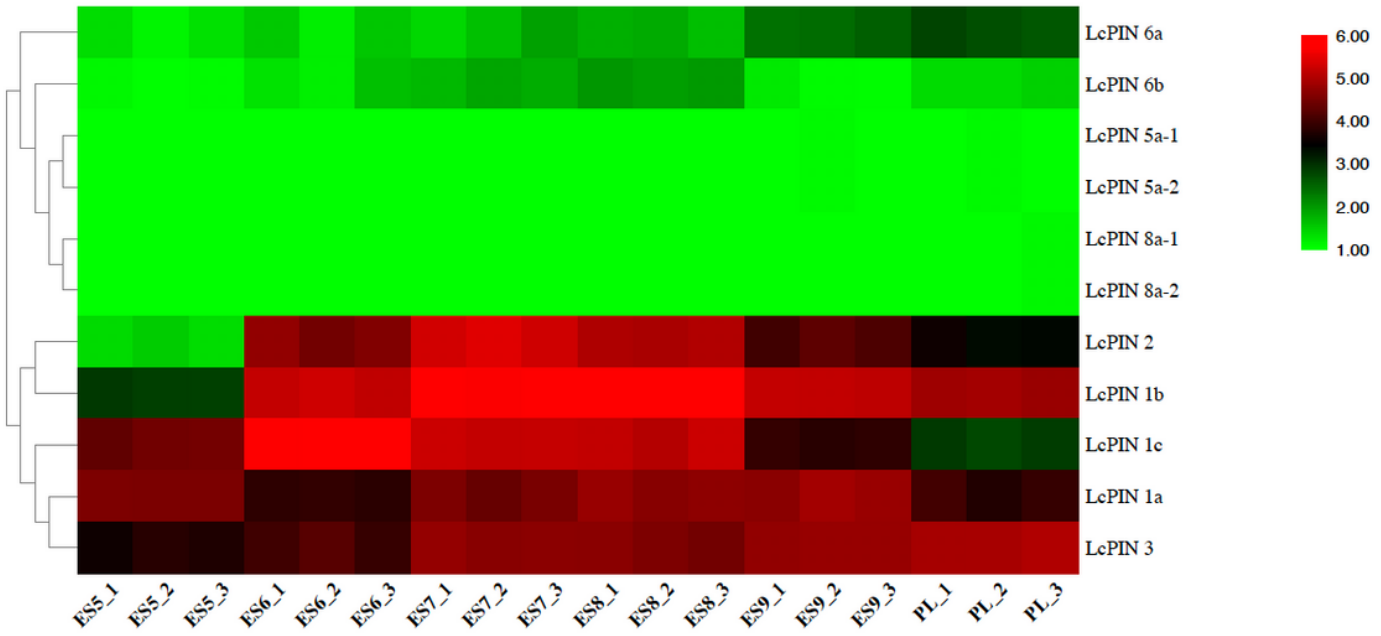

Figure 6

RNA-seq derived specific expression profiles of LcPIN genes during Liriodendron x sinoamericanum somatic embryogenesis. Reads/Kb/Million (RPKM) normalized values are visualized as a heat map. ES5: Globular embryo, ES6: Heart-shaped embryo, ES7: Torpedo embryo, ES8: immature cotyledon embryo, ES9: Cotyledon embryo, PL: Plantlet

Supplementary Files

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