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Identification and Expression Analysis of the *PIN* and *AUX/LAX* Gene Families in Ramie (*Boehmeria nivea* L. Gaud)

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Abstract: Auxin regulates diverse aspects of growth and development. Furthermore, polar auxin transport, which is mediated by the PIN-FORMED (PIN) and AUXIN1/LIKE-AUX (AUX/LAX) proteins, plays a crucial role in auxin distribution. In this study, six *PIN* and four *AUX/LAX* genes were identified in ramie (*Boehmeria nivea* L.). We used qRT-PCR to characterize and analyze the two gene families, including phylogenetic relationships, intron/exon structures, cis-elements, subcellular localization, and the expression patterns in different tissues. The expression of these genes in response to indole-3-acetic acid (IAA) treatment and drought stress was also assessed; the results indicate that most of the *BnAUX/LAX* and *BnPIN* genes were regulated as a result of IAA treatment and drought stress. Our study provides insights into ramie auxin transporters and lays the foundation for further analysis of their biological functions in ramie fiber development and adaptation to environmental stresses.

Keywords: auxin; AUX/LAX; PIN; ramie; expression pattern; drought stress

1. Introduction

Auxin is a phytohormone that controls numerous aspects of plant growth and developmental processes, including apical dominance [1], phloem and wood formation [2,3], flower abscission [4], fruit and root development [5,6], phototropism [7], and leaf formation [8]. In addition, auxin participates in plant responses to abiotic stresses [9,10]. Indole-3-acetic acid (IAA) is the main form of auxin in plant hormones. There are two distinct pathways of auxin transport in plants: passive transport through phloem and active intercellular transport. Auxin influx and efflux carriers promote the intercellular movement of auxin [11]. Polar auxin transport (PAT), combined with local auxin biosynthesis, plays an important role in maximizing auxin production, and is essential for plant development and stress responses [12,13]. The interaction and coordination of auxin influx and efflux carrier proteins in plants constitute a flexible network that can respond to environmental and developmental changes. The four known auxin transporter families in plants are the PIN family, PIN-LIKES (PILS) family, AUX/LAX family, and ATP-binding cassette family B (ABCB)-P-glycoprotein (PGP) family [14,15]. Among these four families, AUX/LAX and PIN are the most well-characterized families involved in auxin influx and auxin efflux.

AUX/LAX proteins transport auxin into cells [16]. In *Arabidopsis thaliana*, the *AUX/LAX* family consists of four highly conserved genes: *AUX1*, *LAX1*, *LAX2*, and *LAX3* [17]. *AUX/LAX* genes encode multimembrane-spanning transmembrane proteins, and biochemical and genetic evidence suggests

that members of the *AUX/LAX* family are functional auxin influx carriers and mediate auxin-related developmental programs in different tissues and organs [18]. From early embryonic development, cellular patterning requires AUX1/LAX-dependent auxin influx, and the expression of *AUX1* and *LAX2* is controlled by the MONOPTEROS-BODENLOS (MP-BDL) signaling pathway [16]. *AUX1* is expressed in tissues related to gravity perception, signal transmission, and signal response [18,19], while *LAX2* plays an important role in normal xylem development [20]. In addition, *aux1* and *lax1* mutants had significantly altered leaf phyllotaxy [21], *lax1* and *lax3* mutants had reduced lateral root formation [20,22], and the *lax2* mutant had vascular breaks in cotyledons [17].

The auxin efflux carriers in the PIN family transport auxin throughout the plant in a polar manner [23–25]. PIN proteins are usually located in the plasma membrane (PM) or endoplasmic reticulum (ER) to direct the auxin flow. The first *PIN* family member was identified in Arabidopsis in association with auxin transport [26]. To date, eight PIN protein family members, named PIN1–PIN8, have been isolated from Arabidopsis [27]. Among them, the PIN1–4 and PIN7 proteins are localized in the PM and function as auxin efflux carriers. The PIN5, PIN6, and PIN8 proteins have a reduced hydrophilic loop in the middle which may regulate the auxin exchange between the cytosol and ER. Variations in the activity of these genes cause altered levels of free IAA and IAA conjugates and affect nuclear auxin signaling [13]. PINs have functional redundancy, and their biochemical activity is regulated in multiple stages [27]. Furthermore, the fewest *PIN* genes are found in *Marchantia polymorpha*, which has 4, while the most are found in *Glycine max*, which has 23 [28]. In Arabidopsis, *PIN1* is involved in floral bud [26] and leaf shape formation [29], shoot vascular development [30], gravitropic and phototropic responses [31,32], and vein patterning [29]. *PIN2* is expressed in cortical and epidermal cells of apical elongation zones [33,34] and is involved in the root gravitropic response [35]. *PIN3* is involved in lateral root formation [36], apical hook formation and maintenance [37], phototropic responses [32], and gravitropism [38]. *PIN4* plays roles in phototropic responses and apical hook development [35], and is expressed in the meristems of roots [39]. *PIN5* is involved in early embryogenesis, cotyledon expansion, lateral root initiation, and root and hypocotyl growth [40]. *PIN6* is dually localized in the PM and ER. It regulates intracellular auxin homeostasis and auxin transport during plant growth, including shoot apical dominance, adventitious root formation, root waving, root hair growth, and lateral root primordia development [41,42], and also participates in inflorescence stem elongation [43], production of nectar, and short stamen development [23]. *PIN7* participates in gravitropic and phototropic responses [32,44], early embryogenesis [25], and apical hook development [45]. *PIN8* acts as a pollen-specific auxin carrier, and is involved in sporophyte and male gametophyte development [28,46,47].

Although extensive research has been conducted on the *AUX/LAX* and *PIN* gene families in species throughout the plant kingdom, including Arabidopsis, *Populus*, *Glycine max*, *Sorghum bicolor*, *Zea mays*, *Capsicum annuum*, and cotton (*Gossypium hirsutum*) [47–53], little is known about these genes in ramie. Ramie has been cultivated for more than 4,700 years in China. The ramie fiber made from stem bast is an excellent textile material that is widely used in industrial fabrics and the manufacture of garments. Moreover, ramie is used as a forage crop in the south of China [54]. The present study provides comprehensive information about the *BnAUX/LAX* and *BnPIN* gene families. Gene identification and structure, basic parameters, phylogenetics, promoter cis-regulatory element analysis, tissue expression patterns, transcriptional responses to hormone treatment and abiotic stress, and subcellular localization are addressed. The results of this study could provide a foundation for further research.

2. Materials and Methods

2.1. Plant Materials, Treatments, and Sampling

Ramie cv. 1504 was planted in the ramie germplasm repository of the Huazhong Agricultural University (Wuhan, Hubei Province, China). The shoots, leaves, stem bark, and roots of 2-month-old plants were sampled. For indole acetic acid (IAA) treatment, the tips of young shoots (about 15 cm)

were cut, and the incisions were immersed in 0.1 g/L $KMnO_4$ for 2 days and then cultured in water for rooting. Afterward, all plants were transferred to Hoagland's nutrient solution for 7 days. Some plants were then treated with 0.05 M IAA (Sigma-Aldrich, Saint Louis, MO, USA), while others continued to grow in Hoagland. After 60 min of IAA treatment, the leaves were sampled, immediately frozen in liquid nitrogen, and stored at −80 °C. There were three biological replicates for each sample.

2.2. Identification of BnPIN and BnAUX/LAX Auxin Transporter Gene Families in Ramie

The *AtPIN* and *AtAUX/LAX* gene sequences were obtained from the TAIR database [55]. Because mulberry (*Morus notabilis*) and ramie both belong to the order Urticales, the *MnPIN* and *MnAUX/LAX* gene sequences were downloaded from the mulberry genome database [56]. All the obtained sequences of the two gene families were used to search three ramie transcriptome databases [57–59]. ClustalX [60] was used to align the sequences from the three transcriptome databases according to the nucleotide sequence. If two or more sequences from different databases overlapped partially (more than 50 bp) or completely, they were further assembled. Finally, all the aforementioned genes obtained were analyzed by using the Open Reading Frame Finder [61] to obtain the coding sequences (CDSs), which were submitted to GenBank [62] (Table 1). The genome sequences of the *BnPIN* and *BnAUX/LAX* gene families were obtained using the CDSs to conduct a BLASTN search in the two ramie genome databases [54,63].

Table 1. Auxin transport-related genes in ramie and their CELLO localizations.

Gene	GenBank Number	CDS (bp)	Predicted Protein Length (aa)	Molecular Weight	Theoretical pI	CELLO Localization
BnAUX1	KR139986	1470	489	54960.83	8.41	PlasmaMembrane (4.989)
BnLAX1	KR139987	1467	488	54610.79	8.77	PlasmaMembrane (4.989)
BnLAX2	KR139988	1491	496	55944.47	9.01	PlasmaMembrane (4.970)
BnLAX3	KR139989	1410	469	53098.19	9.18	PlasmaMembrane (4.970)
BnPIN1a	KR139990	1776	591	63313.03	8.70	PlasmaMembrane (4.225)
BnPIN1b	KR139991	1869	622	67970.12	8.64	PlasmaMembrane (4.601)
BnPIN3	KR139992	2022	673	72588.55	7.30	PlasmaMembrane (3.607)
BnPIN5	KR139993	1086	361	39707.95	6.30	PlasmaMembrane (3.607)
BnPIN6	KR139994	1656	551	60102.28	8.96	PlasmaMembrane (3.607)
BnPIN8	KR139995	1080	359	39279.19	9.40	PlasmaMembrane (3.607)

2.3. Phylogenetic Analysis, Gene Structure, and Protein Profile Analysis

In this study, phylogenetic relationships were constructed with all the BnAUX/LAX and BnPIN amino acid sequences of Arabidopsis, mulberry, and ramie using the neighbor-joining (NJ) method in MEGA software (version 5.0), and the NJ tree was evaluated by 1000 bootstrap replicates [64]. Conserved functional domains in the protein sequences were analyzed by online MEME software (version 5.0.4) [65]. Protein transmembrane topology was predicted using TMHMM Server (version 2.0) [66]. The protein lengths, molecular weights, and theoretical isoelectric points were analyzed by the online ProtParam tool of ExPASy server [67]. Protein subcellular localization was predicted online by CELLO (version 2.5) [68].

2.4. Cis-Elements in the Promoter Regions of BnAUX/LAX and BnPIN Genes

The cis-elements in the *BnPIN* and *BnAUX/LAX* gene promoter regions were surveyed by searching the ramie genome database to retrieve 2 kb sequences that are upstream of the initiation codon. The putative cis-acting elements associated with stress responses, growth, and development were identified online by PlantCARE [69]. The image data were displayed using TBtools software (version 0.6652) [70].

2.5. RNA Extraction and Real-Time Quantitative PCR Analysis

RNA was extracted using the RNA Prep Pure Plant kit (Tiangen Biotech, Beijing, China) and then reverse-transcribed by the GoScript Reverse Transcription System (Promega, Madison, MI, USA). Quantitative real-time PCR was performed on a Bio-Rad iQ5 Real-Time PCR System (Bio-Rad, Hercules, CA, USA). The glyceraldehyde-3-phosphate-dehydrogenase (*GAPDH*) gene was selected as the internal control [58]. Specific primers were designed online (http://primer3.ut.ee/) (Table S1). The 20 µL reaction system included 1 µL of cDNA, 1 µL of forward primers, 1 µL of reverse primers, 10 µL of iTaq Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA), and 7 µL of ddH_2O. The thermocycling regime consisted of 5 min at 95 °C, 40 cycles of 15 s at 95 °C, and 30 s at 60 °C. Each sample was replicated three times. The data were calculated by the $2^{-\Delta\Delta Ct}$ method [71].

2.6. Expression Profiling of BnAUX/LAX and BnPIN Genes

To further investigate the *BnAUX/LAX and BnPIN* expression, RNA-Seq data (measured by normalized FPKM) including bast fiber development (top, middle, and bottom) [57], in vitro organogenesis (W0, W1, W2, W3, W4) [58], and drought stress (1, 24, 72 h) [59] was used for analysis. These data were presented in heat maps using the R software (version 3.6.0).

2.7. Subcellular Localization of BnAUX/LAX and BnPIN Proteins

To further confirm the subcellular localization of the BnPIN5 and BnAUX/LAX proteins, we constructed a BnPIN (AUX/LAX):GFP fusion gene controlled by the CaMV35S promoter (refer to Figure 7a). Specific primers were designed from both ends of the selected sequence (Table S2). Then, the fusion genes and empty vector (positive control) were transformed into tobacco (*Nicotiana benthamiana*) by Agrobacterium-mediated infiltration as described previously [72]. After transient transformation, the tobacco plants were grown in the dark for 24–48 h at room temperature, and then the epidermal cells were examined by a laser scanning confocal microscope (Olympus FV1200, Japan), the green fluorescence was excited with a 488-nm laser line, and cells were detected using a NIBA emission filter. The epidermal cells of untreated tobacco leaves were also examined as negative controls. The images were processed by Adobe PhotoshopCC2017.

3. Results

3.1. Identification and Phylogenetic Analysis of Ramie BnAUX/LAX and BnPIN Families

In total, four BnAUX/LAX and six BnPIN genes were identified in ramie (Table S3). From the phylogenetic tree, the BnLAX gene family can be divided into two subfamilies (Figure 1a). BnAUX1 and BnLAX1 belong to subfamily I, and BnLAX2 and BnLAX3 belong to subfamily II. A total of 20 PIN proteins, including 6 BnPIN, 8 AtPIN, and 6 MnPIN proteins, were used to construct a phylogenetic tree (Figure 1b). The BnPIN family was divided into four subgroups. BnPIN3 belongs to subgroup I, BnPIN1a and BnPIN1b belong to subgroup II, BnPIN6 and BnPIN8 belong to subgroup III, and BnPIN5 belongs to subgroup IV. Moreover, most BnAUX/LAX and BnPIN proteins were more similar to those in mulberry compared with those in Arabidopsis.

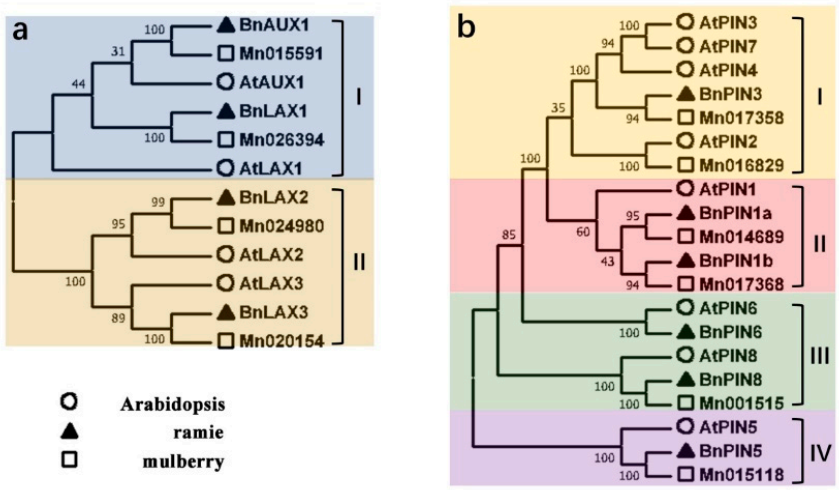

Figure 1. Phylogenetic relationship analysis of the (**a**) LAX and (**b**) PIN families among ramie, Arabidopsis, and mulberry. All branches are marked with bootstrap values. Different colored boxes indicate different subfamilies.

3.2. Phylogenetic, Protein Domain, and Gene Structure Analysis of the BnAUX/LAX and BnPIN Families

The conserved motifs of the AUX/LAX and PIN proteins were investigated by MEME (Figure 2). The AUX/LAX protein sequences have a conserved domain with a length of more than 400 amino acids (Figure 2a). Ramie PIN proteins have two conserved domains of 170 and 161 amino acids, respectively (Figure 2b). The CDS of *BnAUX/LAX* varies between 1410 and 1491 bp, coding 469–496 amino acids; the molecular weight is 53098.19–55944.47, and the isoelectric point (*pI*) is 8.41–9.19. TMHMM2 software predicted 10 transmembrane helices in BnAUX/LAX proteins. The CDS of *BnPIN* varies between 1086 and 2022 and encodes 359–673 amino acids; the molecular weight is 39279.19–72588.55, and the pI is 6.30–9.40. The transmembrane helices of BnPIN proteins range from 8 to 10 (Table 1, Figure 3), and all PINs contain two hydrophobic domains separated by a hydrophilic loop. There are 6, 6, and 8 exons in *BnLAX1*, *BnLAX2*, and *BnLAX3*, respectively, and 7, 7, 6, 7, 6, and 4 exons in the *BnPIN* genes. The difference between the longest gene *BnPIN6* (with a gene size of 12.9 kb) and the shortest gene *BnPIN8* (1.9 kb) is mainly due to the total intron length (Figure 4).

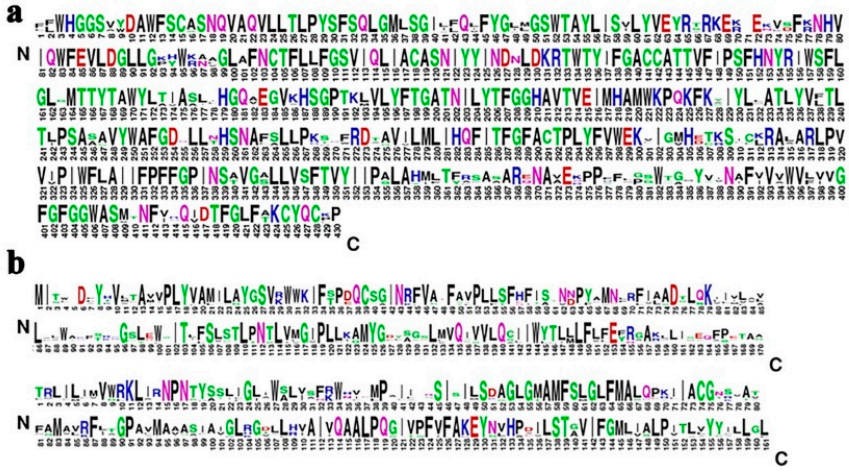

Figure 2. Conserved domains of (**a**) AUX/LAX and (**b**) PIN proteins in ramie. The symbol heights indicate the relative frequency of each amino acid at that position.

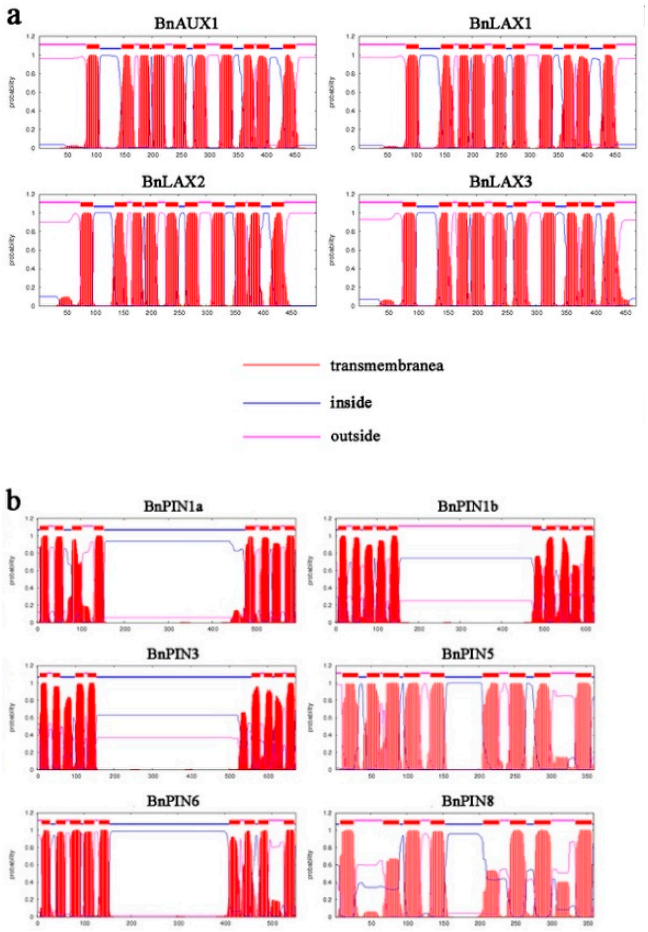

Figure 3. Transmembrane topology analysis of the (**a**) BnAUX/LAX and (**b**) BnPIN proteins in ramie. The predicted transmembrane helices are shown as red peaks.

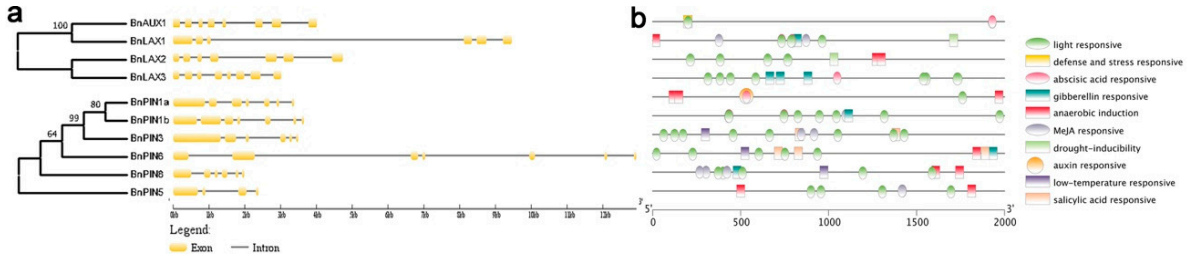

Figure 4. The (**a**) gene structure and (**b**) cis-element prediction of *BnAUX/LAX* and *BnPIN* promoters. The exons are represented by yellow boxes, and the introns are represented by lines.

3.3. Cis-Element Prediction in BnAUX/LAX and BnPIN Promoters

Cis-acting elements that are bound by transcription factors and involved in plant stress response, growth, and development [73], among other processes. Promoter cis-element analysis reveals several phytohormone-related and stress-related motifs in the *BnAUX/LAX* and *BnPIN* gene promoter regions (Figure 4, Table S4). Ten common cis-regulatory elements are briefly characterized as auxin-responsive, MeJA-responsive, salicylic acid-responsive, gibberellin-responsive, defense- and stress-responsive, abscisic acid-responsive, anaerobic-inducible, and drought-inducible elements. Furthermore, light-responsive elements are pervasive. These results indicate that the *BnAUX/LAX* and *BnPIN* genes are vital to various hormone signaling and abiotic stress responses, which might be hypothesized by their diverse natures.

3.4. Tissue-Specific and Treatment-Induced Expression Profiles of BnAUX/LAX and BnPIN

The tissue-specific expression levels of the BnAUX/LAX and BnPIN genes are shown in Figure 5. BnAUX1, BnLAX2, BnPIN3, BnPIN5, BnPIN6, and BnPIN8 were highly expressed in the leaves, while BnPIN1b had high expression levels in the bark. BnLAX1, BnPIN5, BnPIN6, and BnPIN8 were expressed at relatively low levels in four tissues. After IAA treatment, the relative expression of BnAUX1, BnLAX1, BnPIN1b, BnPIN5, and BnPIN8 decreased. Conversely, the relative expression of BnLAX2, BnPIN3, and BnPIN6 increased, and there was no significant change in the relative expression of BnLAX3 and BnPIN1a (Figure 5).

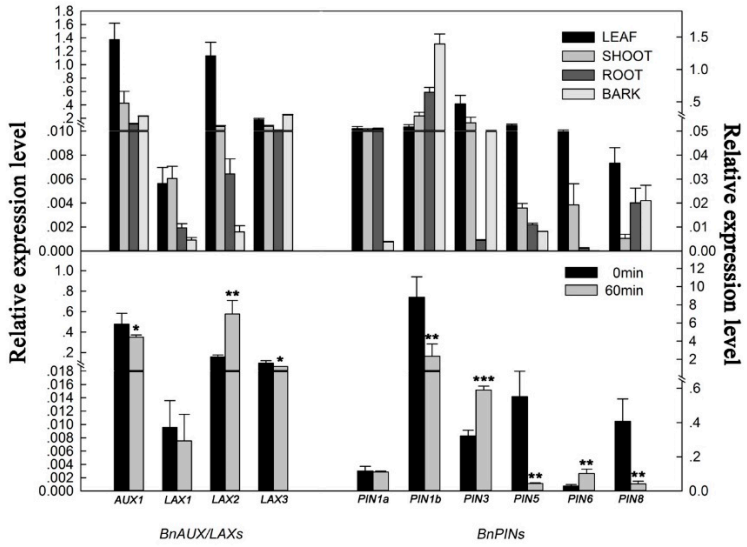

Figure 5. Expression pattern of (**a**) *BnAUX/LAX* and *BnPIN* genes in ramie tissues (leaf, shoot, root, bark) and (**b**) before and after IAA treatment (0 and 60 min). (* *t*-test, *p*-value < 0.1, ** *p*-value < 0.01, *** *p*-value < 0.001). The error bar represents the standard error.

In bast fiber development, expression of the BnAUX1, BnLAX2, and BnPIN1a genes was inhibited. In the early stage of fiber development (the top part of the stem bark), only BnLAX3, BnPIN1b, and BnPIN3 were highly expressed; in the middle part of the bast fiber, the BnPIN5, BnPIN6, BnPIN8 genes were distinctly upregulated. The bottom part of the bast fiber represents the mature fiber, and the BnLAX3, BnPIN1b, and BnPIN3 genes were expressed to a higher degree relative to others. In vitro organogenesis includes the development of callus and shoot buds during regeneration, and intervals of 0 (W0), 4 (W1), 14 (W2), 28 (W3), and 35 (W4) days (the buds were observed for 30 days) were set on the basis of morphological observation. BnAUX1, BnPIN1a, and BnPIN1b were more expressed than other genes, and BnPIN6 was upregulated. Polyethylene glycol (PEG) treatment for 24 h caused the downregulation of the expression levels of most BnAUX/LAX and BnPIN genes, which were still downregulated after treatment for 72 h. In contrast, in the roots, most BnAUX/LAX and BnPIN genes were upregulated when treated for 24 h, and most genes were downregulated when PEG treatment lasted 24–72 h (Figure 6). It is worth mentioning that 0–24 and 24–72 h PEG treatment caused upregulation of the BnLAX1 gene in the roots for both periods, while the expression level of BnPIN5 was downregulated and then upregulated in the roots.

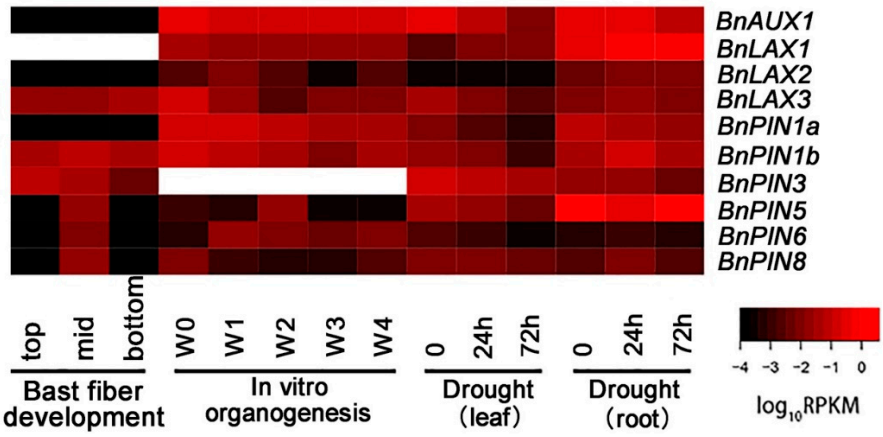

Figure 6. Expression pattern of *BnAUX/LAX* and *BnPIN* genes in ramie by transcriptome analysis (blank spaces represent no data).

3.5. Subcellular Localization of BnAUX/LAX and BnPIN5 Proteins

The positive control and the fusion constructs were transiently transformed into tobacco leaf cells. In Figure 7b, no GFP is observed in the negative control, and the GFP signal is distributed throughout the tobacco leaf cells in the GFP positive control. The GFP signals from the BnPIN5-GFP and BnAUX/LAX-GFP fusion proteins are observed clearly in the membrane, suggesting that the four fusion proteins were localized in the cell membrane.

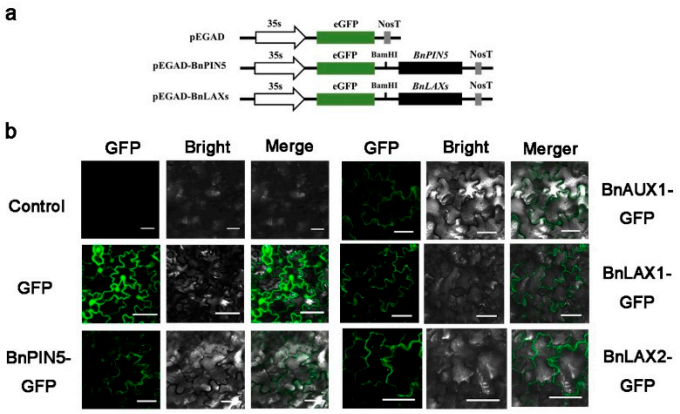

Figure 7. Subcellular localization of BnPIN5, BnAUX1, BnLAX1, and BnLAX2 proteins in tobacco leaf: (**a**) subcellular localization of the fused pEGAD-35S::BnPIN (BnAUX/LAX):GFP in tobacco leaf cells. The pEGAD-35S::GFP construct was used as the control. (**b**) The fusion proteins were transiently expressed in tobacco epidermis cells. Merged overlays of bright-field and green fluorescence images are shown. The scale bars are 20 μm.

4. Discussion

4.1. Characterization and Analysis of BnAUX/LAX and BnPIN Genes in Ramie

Six *PIN* and four *AUX/LAX* genes were identified in ramie, a number of genes that is similar to the number in Arabidopsis. The biological functions of the *AUX/LAX* and *PIN* genes have been revealed in Arabidopsis. Therefore, studying the evolutionary relationships of AUX/LAX and PIN proteins among ramie, mulberry, and Arabidopsis can help us understand the possible biological functions of these genes. The phylogenetic analysis shows that the phylogenetic relationship between ramie and mulberry is closer than that between ramie and Arabidopsis. It is predicted that all BnAUX/LAX and BnPIN proteins are localized in the membrane, and the subcellular localization of the BnAUX1, BnLAX1, BnLAX2, and BnPIN5 proteins in tobacco are located in the membrane. The PIN5 protein

has a reduced hydrophilic ring which is typically located in the internal compartment [40]. However, another study pointed out that the PIN5 protein is clearly localized in the PM [74]. For the *BnAUX/LAX* and *BnPIN* genes, we also explored the cis-regulatory elements in the promoter regions and discovered the enrichment of several hormone- and stress-related cis-elements, as well as many light-responsive elements (Table S4). The prediction of the cis-regulatory elements indicates that that *BnAUX/LAX* and *BnPIN* genes may participate in the drought stress response and drought tolerance. In Arabidopsis, PIN play important roles in regulating asymmetrical auxin translocation during phototropism [38]. Among them, *PIN3* regulates the lateral translocation of auxin and plays a role in gravitropism and phototropism [38,75].

4.2. Analyses of Tissue-Specific Expression of BnAUX/LAX and BnPIN Genes

The differential expression of most *BnAUX/LAX* and *BnPIN* genes in tissues indicates that they may be involved in the regulation of ramie growth and development. Nearly all the *BnAUX/LAX* and *BnPIN* genes are highly expressed in the leaves. In Arabidopsis, the vein patterning in leaf is controlled by two distinct auxin transport pathways: *PIN1*-mediated intercellular auxin transport in the PM and *PIN6*-, *PIN8*-, and *PIN5*-mediated intracellular auxin transport in the endoplasmic reticulum [76]. Moreover, phyllotaxis changes when *AUX1/LAX* activity is lost [23]: the quadruple mutant *aux1 lax1 lax2 lax3* and the single mutants *aux1*, *lax2*, and *lax3* exhibit enhanced asymmetry in their venation patterns [20]. Therefore, we infer that the *BnAUX/LAX* and *BnPIN* genes may regulate auxin transport during leaf development. The *BnLAX1*, *BnLAX2*, *BnPIN1a*, *BnPIN5*, and *BnPIN6* genes show low expression levels in the bark. In contrast, *BnPIN1b* is strongly expressed in the bark. In an analysis of *PIN* genes in cotton, fiber elongation was observed when the expression of *PIN* genes was increased [77]. Further research on *BnPIN1b* may increase our knowledge of the molecular mechanisms underlying bast fiber development in ramie.

4.3. BnAUX/LAX and BnPIN Genes Were Responsive to IAA Treatment and Drought Stress

Previous studies have reported crosstalk between auxin and biotic and abiotic stress signaling [78]. To confirm whether the *BnAUX/LAX* and *BnPIN* genes participate in IAA signaling and drought responses, we analyzed the gene expression levels in ramie treated with IAA and PEG. Many *BnAUX/LAX* and *BnPIN* genes responded to IAA treatment and drought stress at the transcriptional level, and they were differentially expressed in leaf and root in response to drought stress. In soybean, most of the *PIN* genes respond to a variety of phytohormone stimuli and abiotic stresses [79]. In sorghum, most of the *SbPIN* genes are upregulated by IAA treatment, and IAA induces *SbLAX2* and *SbLAX3*, but the expression of *SbLAX1* and *SbLAX4* is inhibited in leaf and root [80]. In maize, the expression of most *ZmPIN* and *ZmLAX* genes is upregulated in the shoots, but these genes are downregulated in the roots as a result of drought stress [51]. In rice, the IAA content is reduced after drought stress. In response to these stresses, many genes involved in IAA biosynthesis and signaling change at the transcriptional level, and these changes are basically consistent with changes in the level of endogenous IAA [81]. *OsPIN3t* is involved in auxin transport and the drought stress response, suggesting that the polar auxin transport pathway is involved in regulating plant responses to water stress [82]. The synergistic or antagonistic hormone action and the coordinated regulation of hormone biosynthetic pathways play key roles in plant adaptation to abiotic stresses [83]. The versatile expression responses of *BnAUX/LAX* and *BnPIN* genes to IAA and drought stress suggest that these genes are controlled by complex regulatory networks. This is supported by the prediction analysis of the cis-element in the promoters of *BnAUX/LAX* and *BnPIN*. Drought stress severely affects ramie stem growth, and fiber production is easily affected by an arid environment [84]. *AUX/LAX* and *PIN* in ramie might promote plant adaptation to drought stress by participating in the regulation of auxin distribution.

5. Conclusions

This study comprehensively analyzed the *AUX/LAX* and *PIN* genes in ramie. Further research, such as the identification of biological functions and genetic analysis of each *BnAUX/LAX* and *BnPIN* gene, will accelerate the study of the molecular mechanisms mediated by auxin transporters that regulate fiber development and abiotic stress tolerance. The results of such studies can be used to increase the yields of ramie fiber and enhance the resistance to various stresses, thus improving plant performance.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/9/8/435/s1, Table S1: Primers for qRT-PCR analysis; Table S2: Primers for subcellular localization; Table S3: Gene sequences of *BnAUX/LAX* and *BnPIN* from three databases; Table S4: Cis-Element Prediction of *BnAUX/LAX* and *BnPIN* Promoters.

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