Genome-wide identification and expression analysis of ClLAX, ClPIN and ClABCB genes families in Citrullus lanatus under various abiotic stresses and grafting

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Abstract

Background: Auxin plays an important role in regulating plant growth and development as well as in the response of plants to abiotic stresses. Auxin is transported by three kinds of major protein families, including the AUXIN RESISTANT 1/LIKE AUX1 (AUX/LAX) influx carriers, the PIN-FORMED (PIN) efflux carriers and the ATP binding cassette B/P-glycoprotein/Multidrug-resistance (ABCB/MDR/PGP) efflux/condition carriers. The biological function of several auxin transporter genes has been well characterized in Arabidopsis thaliana. However, their function in response to exogenous auxin and abiotic stresses in watermelon (Citrullus lanatus L.) remained unknown.

Results: Here, the latest updated watermelon genome was used to characterise the ClLAX, ClPIN and ClABCB family genes from watermelon. The genome-wide analysis of the ClLAX, ClPIN and ClABCB family genes, including chromosome localisation, gene structure, and phylogenic relationships, was carried out. Seven ClLAXs, 11 ClPINs and 15 ClABCBs were mapped on 10 watermelon chromosomes. The expression profiles of the ClLAX, ClPIN and ClABCB genes under exogenous indole-3-acetic acid and various abiotic stresses (salt, drought, and cold stresses) treatments were performed by quantitative real-time PCR (qRT-PCR). The transcriptional level of majority ClLAX, ClPIN and ClABCB genes were changed by abiotic stresses in both shoots and roots. We also analysed the expression levels of ClLAX, ClPIN and ClABCB genes in graft response.

Conclusion: Analysis of the expression patterns of ClLAX, ClPIN and ClABCB genes under salt, drought, cold treatment and grafting response helps us to understand the possible roles of auxin transporter genes in watermelon adaptation to environmental stresses.

Keywords: ABCB, Abiotic stresses, Grafting, LAX, PIN, Watermelon

Background

Auxin is a very important plant hormone involved in regulating many processes of plant growth and development, such as root formation, apical dominance, inflorescence and phyllotaxy development, vascular tissue differentiation, fruit maturation and responses to illumination and gravity. Abnormal phenotypes are observed in plants, which are caused by excessive or insufficient concentrations of endogenous auxin [1]. Plants are inevitably subject to abiotic stresses such as salinity, cold, high temperature and drought during the life cycle.Auxin plays a key role in plant response to stress [2, 3] and environmental stress response relies on auxin homeostasis within different plant tissues [4]. The homeostasis of auxin is often disturbed by abiotic stress, which leads to the change of plant growth and development [5, 6].

Auxin is primarily synthesised in apical meristems and developing leaf tips, then transported to distal target tissues either through the bulk flow in stem vascular tissues in a non-polar free diffusion or actively in a polar transport [7]. Auxin transport exhibits polarity, which is unique among all phytohormones. The polar transport...
of auxin is mediated through the auxin carriers, including AUXIN RESISTENT1/LIKE AUX1 (AUX/LAX) influx carrier, PIN-FORMED (PIN) efflux carriers, and ATP binding cassette B/P-glycoprotein/Multi-drug-resistance (ABC/PDR/PGP) efflux condition carriers [8–10].

AUX/LAX family is a subclass of amino acid superfamily recognized as auxin input carrier family. AtAUX1 is the first AUX/LAX family gene cloned in Arabidopsis, which encoded a protein containing 11 transmembrane structure [8]. Mutations of AUX/LAX show the auxin-related developmental defects in Arabidopsis thaliana. Ataux1 mutants are gravitropic and selective resistant to auxin [11]. They are insensitive to indole-3-acetic acid (IAA) and (2, 4-dichlorophenoxy)-acetic acid (2, 4-D). Only free diffusion of naphthalene-1-acetic acid (NAA) can restore the gravitropism of ataux1 [11, 12]. AtLAX3 and AtAUX1 co-ordinately regulate lateral root development by regulating the emergence and initiation of lateral root primordia [13, 14]. AtAUX1 and AtLAX3 are high-affinity auxin transporters by auxin uptake experiments in heterologous expression systems [13, 15, 16]. Disruption of the AtLAX2 gene results in increasing division of the cells in the quiescent centre (QC) and decreasing expression of AtWOX5 and the auxin response reporter DR5 [17]. The AUX LAX family gene affects phyllotactic patterning and is needed to establish the embryonic root cell organization and plant embryogenesis in Arabidopsis [18, 19]. PaLAX1, from wild cherry (Prunus avium), promotes the absorption rate of auxin in cells and affects the distribution of free endogenous auxin [20]. OsAUX1 controls the lateral root initiation, primary root and root hair elongation in rice [21, 22]. In sorghum, maize (Zea mays) and soybean (Glycine max), some AUX/LAX genes are in response to hormonal and abiotic stress at transcriptional level [23–25].

Among the auxin carriers, PIN family is extensively studied in Arabidopsis. The PIN family was first cloned and comprised of eight members in Arabidopsis [26]. The PIN family genes play crucial roles in various aspects of developmental processes, including root meristem patterning, root hair growth, lateral root development, vascular bundle differentiation, phototropism and embryo development [27–29]. PIN proteins are localised either on the plasma membrane (AtPIN1, −2, −3, −4 and −7) or in the endoplasmic reticulum (ER) (AtPIN5, AtPIN6 and AtPIN8). PIN proteins also play a vital role in both intracellular and intercellular auxin homeostasis [30, 31]. The PIN efflux transporter asymmetric localisation on the plasma membrane regulates the direction of the flow of auxin [32]. For example, AtPIN1 is asymmetrical localised on the basal rootward face of vascular cells [33]. The study of the PIN family has been expanded to other species not limited to Arabidopsis. In maize (Zea mays), two putative orthologues of AtPIN1, ZmPIN1a and ZmPIN1b, have been analysed involving in endosperm and embryonic development [34, 35]. In rice (Oryza sativa), as the closest orthologue of AtPIN1, OsPIN1b is been detected expressed in the roots, stem base, stem, leaves and young panicles [36, 37]. By analysis the phenotype of overexpression and RNAi lines, OsPIN1b may involve in auxin transport in primary and adventitious roots in rice [36]. The auxin transport from the shoot to the root–shoot junction is increased in OsPIN2 overexpression plants. Overexpression of OsPIN2 resulted in a larger tiller angle, a lowered plant height and an increased tiller number compared with the wild type [38]. A putative auxin efflux carrier of rice, OsPIN3t, is involved in the drought stress response and drought tolerance [39]. Three monocot-specific PIN genes from rice, OsPIN9, OsPIN10a, and OsPIN10b, are expressed at high level in adventitious root primordia and pericyclic cells at the stem base, suggesting that they might be involved in adventitious root development [37].

The ATP-binding cassette (ABC) superfamily contains more than 100 members in plants [40]. The subfamily B (ABC/B), previously known as multidrug resistance (MDR)/phospho-glycoprotein (PGP) proteins, some of them are involved in auxin transport [41, 42]. Six members of ABCB transporters in Arabidopsis (AtABC1B, −4, −14, −15, −19 and −21) have been associated with auxin transport [41–44]. To date, AtABC1B, AtABC4 and AtABC19 are the best characterised ABCBs. Both AtABC1B and AtABC19 are involved in auxin efflux. AtABC1B and AtABC19 coordinate with AtPIN1 in long distance transport of auxin along the plant main axis, and regulate root and cotyledon development [45–47]. AtABC4 and AtABC21 function as an efflux and influx carrier that controls cellular auxin levels [44, 48]. AtABC14 was first described as a malate importer modulating stomata aperture response to CO2 levels [49]. AtABC14 and AtABC15 are expressed in vascular tissues of primary stem by promoter::glucuronidase reporter assays. Anatomical alterations of the vascular tissue of the primary stem have been shown and IAA transport along the inflorescence is reduced in both atabc14 and atabc15 mutants, these results suggesting AtABC14 and AtABC15 might participate in auxin transport [43]. OsABC14, a rice gene high homology with AtABC14, has been demonstrated as an auxin influx transporter, and its knockout mutants are insensitive to 2, 4-D and IAA. OsABC14 was found to be involved in iron homeostasis in rice [50].

Recently, auxin transporter genes have been studied throughout the plant kingdom, such as Medicago sativa, Glycine max, Populus trichocarpa, Prunus avium, Oryza sativa, Sorghum bicolor, and Zea mays [20, 23–25, 51]. However, little or nothing is known about the LAX, PIN
and ABCB families in watermelon (Citrullus lanatus) to date. Watermelon is an important cucurbit crop and its output value accounted for more than 10% of the total output value of the vegetable industry in China. Watermelon seedling’s growth stops below 10 °C and cannot survive below the 1 °C [52]. Salinity and drought are the major environmental stresses in plant agriculture worldwide. Grafting is widely used to improve plants adaptation to biotic or abiotic stress [53, 54]. However, the expression of auxin transporter genes underlying grafting processes remains unclear. In this study, we provide comprehensive information on the CLLAX, CIPIN and CIABCB gene families and expression patterns of those genes exposed to salt, drought and cold stresses. The distinctive tissue-specific expression patterns of the CLILAX, CIPIN and CIABCB genes, and their differential responses to salt, drought and cold stresses are the molecular basis to increase abiotic stress tolerance in watermelon. Our study also provide a new insight into the expression of CLLAX, CIPIN and CIABCB gene families at the phase of grafting.

Methods

Plant material, growth conditions and stress treatments
Watermelon “zaojia” was selected in this study. Seeds were sown in perlite beds after sterilized with 10% sodium hypochlorite for 30 min. Seedlings at the two-leaf were irrigated by half-strong Hoagland solution (pH5.6). The growth conditions were as follows: a 12 h photoperiod under fluorescent light (600 μE m⁻² s⁻¹) at with 60% relative humidity, and temperature of 28/18 °C (day/night). A month old seedlings were used for stress treatment.

For auxin treatment, the roots of watermelon seedlings were soaked in half-strong Hoagland nutrient solution containing 100 μM IAA. For salt stress experiment, the roots of seedlings were immersed in nutrient solution containing 200 mM NaCl. For drought stress experiment, the roots of seedlings were immersed in nutrient solution containing 20% (W/W) PEG6000 (Polyethylene glycol). For cold treatment, seedlings were transferred to a 4 °C growth chamber. Then root and shoot samples of watermelon seedlings at different treatment time points were harvested. For graft experiment, watermelon plants when cotyledon had expanded were grafted onto squash per- top approach grafting method. Experiment was repeated for 3 times with similar results.

For tissue-specific expression analysis, roots, stems, leaves, and cotyledons samples were harvested from two-leaf stage; for flower samples, flowers were harvested at 2 d after opening.

Identification of CLLAX, CIPIN and CIABCB auxin transporter family genes in watermelon
The sequences of CLLAX, CIPIN and CIABCB were collected by homology screening against Cucurbit Genomics Database (http://www.icugi.org/cgi-bin/ICuGI/index.cgi) (version 1). The known sequences of AtLAX, AtPIN and AtABCB were used as queries. The hidden Markov model profiles were used to identify LAX, PIN and ABCB proteins from the proteome of watermelon. Pfam 01490 (Transmembrane amino acid transporter protein) was used for CILAX proteins identification; Pfam 03547 (Membrane transport protein) was used for CIPIN proteins identification; Pfam 00005(ABC transporter) and Pfam 00664 (ABC transporter transmembrane region) were used for CIABCB proteins identification. Protein molecular weight and isoelectric point were predicted by DNastar tool (http://www.dnastar.com/). The transmembrane helices of CLLAX, CIPIN and CIABCB proteins were predicted by TMHMM2 Software (http://www.cbs.dtu.dk/services/TMHMM/).

Quantitative real time-polymerase chain reaction PCR (qRT-PCR)
Total RNA were extracted from 0.1 g of samples using MiniBEST Plant RNA Extraction Kit (code: 9769, TAKARA, JAPAN) according to the manufacturer’s instruction. The primers sequences of qRT-PCR are listed in Additional file 1: Table S1. Quantitative RT-PCR was performed on LightCycler480 instrument (Roche) according to the manufacturer’s instructions. The CIACTIN (Cla004014) was used as internal standards basing on the comparative cycle threshold (2⁻ΔΔCt) values. Heat map was performed by MeV software using the average Ct value to visualize the tissues-specific expression data. All the expression analyses were carried out with three biological repeats.

Results

Genome-wide identification of CLLAX, CIPIN and CIABCB genes in watermelon
In the present study, we used the AUX/LAX, PIN and ABCB full-length protein sequences from Arabidopsis as...
BLAST queries to search Cucurbit Genomics Database (http://www.icugi.org/). Four hidden Markov model profiles (Pfam 01490, Pfam 03547, Pfam 00005 and Pfam 00664) were used to identify the CILAX, ClPIN and CIABCB proteins. Totally seven CILAX genes, 11 CIPIN genes and 15 ClABCB genes were identified. We named them based on their order on the chromosomes. Information on CILAX, CIPIN and ClABCB gene families, including gene names, locus ID, open reading lengths, exon numbers, chromosome locations and deduced polypeptide parameters, were listed in Table 1.

The sizes of the ORF for the CILAX genes ranged from 1326 bp (CILAX3) to 1470 bp (CILAX1), and the sizes of the corresponding proteins were between 441 and 489 amino acids. The molecular masses of CILAX protein varied from 49.5 kDa (CILAX6) to 54.96 kDa (CILAX1). The predicted isoelectric points ranged from 5.453 (CIPIN3) to 10.852 (CIPIN8). The sizes of the ORF for the ClABCB genes ranged from 3504 bp (ClABCB5) to 4368 bp (ClABCB9), and the sizes of the corresponding proteins are between 1167 and 1455 amino acids. The molecular masses of CIABCB protein varied from 127.97 kDa (ClABCB5) to 159.17 kDa (ClABCB9). The predicted isoelectric points varied from 6.722 (ClABCB13) to 9.286 (ClABCB5).

**Chromosomal distribution of CILAX, CIPIN and ClABCB genes**

Based on position of CILAX, CIPIN and ClABCB genes on the watermelon chromosomes, we mapped all 33 genes of CILAX, CIPIN and ClABCB family on chromosomes (Fig. 1a, Table 1). The 33 genes were unevenly distributed on 10 out of the 11 watermelon chromosomes. Among 33 genes, not a single gene was located on chromosome 8. Chromosome 3 only contained one gene. Two genes were located on chromosome 1, 6 and 11, respectively. Three genes were distributed on each of chromosomes 4 and 7. Seven genes were located on chromosome 2 (Fig. 1a). In many plants, including *S. bicolor*, *Arabidopsis*, *G. max*, *O. sativa*, some of the auxin transporter genes were clustered. Three small gene clusters were identified in accord with the definition of gene clusters [55]. Two gene clusters were distributed on chromosome 2 (Fig. 1a). The other one was distributed on chromosome 7. The first gene cluster contained two CIABCB genes (ClABCB2 and ClABCB3). The second gene cluster contained two CIPIN genes (CIPIN2 and CIPIN3). The third gene cluster contained two CIPIN genes (CIPIN8 and CIPIN9).

Gene duplication is the main contributor to evolutionary momentum [56]. The duplication patterns of CILAX, CIPIN and ClABCB families including tandem and segmental duplications were analyzed to find the expansion of CILAX, CIPIN and ClABCB gene families during the evolutionary momentum. Tandem duplication was observed between ClABCB2 and ClABCB3 (Fig. 1b). CILAX/ClLAX5 gene pairs share high similarity in protein sequences (Additional file 2: Table S2), and were lactation on different chromosomes, indicating that they were segmental duplicated gene pair. Three segmental duplications occurred in the CIABCB family gene: ClABCB4/ClABCB15, ClABCB6/ClABCB8 and CIABCB11/CIABCB14 (Fig. 1b).

**Phylogenetic relationship analysis of the CILAX, CIPIN and ClABCB family genes**

Many studies revealed the biological functions of the auxin transporter genes in *Arabidopsis* [11, 29, 42]. Investigation of the evolutionary relationships of three kinds of auxin transporter proteins between watermelon and *Arabidopsis* helps us to understand the possible biological functions of these auxin carriers in watermelon. Multiple protein sequence alignments of full-length amino acid sequences were carried out using the MEA6g.0 software for phylogenetic analysis with the neighbour-joining method. A total of 11 AUX/LAX proteins, including 7 CILAX proteins and 4 AtLAX proteins were used to build a phylogenetic tree (Fig. 2a). The LAX family could be divided into two subfamilies (subfamily I and subfamily II). Six of them belong to subfamily I (CILAX3, 4, 5, 6, *AtLAX1* and *AtLAX1*). A paralogue gene pair existed in the watermelon LAX family: CILAX4/CILAX6. A total of 19 PIN proteins, including 11 CIPIN proteins and 8 AtPIN proteins were used to construct a phylogenetic tree (Fig. 2b). All the PIN family could be grouped into five subfamilies (subfamily I, II, -III, -IV and -V). Two PIN orthologue gene pairs existed between watermelon and *Arabidopsis*: CIPIN6/AtPIN2 and CIPIN10/AtPIN6. A total of 37 ABCB proteins, including 15 CIABCB proteins and 22 AtABCB proteins were used to construct a phylogenetic tree (Fig. 2c). All the ABCB families could be classified into three subfamilies (subfamily I, subfamily II and subfamily III). Two ABCB orthologue gene pairs were existed between watermelon and *Arabidopsis*: CIABCB14/AtABC11 and CIABCB11/AtABC19. Two paralogue gene pair occurred in the watermelon ABCB family: CIABCB2/CIABCB3 and CIABCB6/CIABCB8.

**Analysis of tissue-specific expression and gene structure of CILAX, CIPIN and ClABCB family genes**

To elucidate the biological roles of different members of the CILAX, CIPIN and ClABCB family in watermelon,
the expression of CILAX, CIPIN and CIABCB genes was investigated in different tissues performed by quantitative real-time polymerase chain reaction (qRT-PCR). Total RNA was extracted from the roots, cotyledons, mature leaves, stems and flowers of watermelon. All transcripts of CILAX, CIPIN and CIABCB family genes were detected in the selected tissues. Most of the CILAX, CIPIN and CIABCB genes showed different tissue-specific patterns across the five tissues. As shown in Fig. 3a, the transcriptional level of the CILAX family gene was the highest in the mature leaves and the lowest in the flowers. CIPIN3 and CIPIN5 were highly expressed in roots. CIPIN1, CIPIN8 and CIPIN11 showed the highest level of expression in mature leaves. Most of CIPIN genes were weakly expressed in the flowers. CIABCB3 was much more highly expressed than any other CIABCB genes in the flowers. The level of expression of CIABCB genes was much higher in the stems than in the flowers. All the expression levels of the CILAX, CIPIN and CIABCB family genes in five tissues are listed in Additional file 3: Table S3.

Table 1 Information on CILAX, CIPIN and CIABCB genes and properties of the deduced proteins in watermelon (Citrullus lanatus)

| Gene   | Locus ID | ORF length (bp) | No. of exons | Chromosome No. | Deduced polypeptid Length (aa) | M w (Da) | pI  | No. of transmembrane |
|--------|----------|-----------------|--------------|----------------|-------------------------------|----------|-----|----------------------|
| CILAX1 | Cla015837| 1470            | 8            | Chr2           | 489                           | 54960.23 | 8.927 | 10                   |
| CILAX2 | Cla020298| 1461            | 8            | Chr2           | 486                           | 54841.35 | 9.258 | 10                   |
| CILAX3 | Cla018110| 1326            | 7            | Chr4           | 441                           | 49895.36 | 8.204 | 8                    |
| CILAX4 | Cla004339| 1467            | 7            | Chr7           | 488                           | 54875.07 | 7.804 | 10                   |
| CILAX5 | Cla017975| 1437            | 7            | Chr10          | 478                           | 53841.09 | 8.331 | 10                   |
| CILAX6 | Cla006581| 1329            | 8            | Chr11          | 442                           | 49504.24 | 8.485 | 8                    |
| CILAX7 | Cla000681| 1443            | 8            | chr0           | 480                           | 54063.26 | 8.532 | 10                   |
| CIPIN1 | Cla003909| 1926            | 6            | chr1           | 641                           | 69903.01 | 7.439 | 9                    |
| CIPIN2 | Cla010530| 1041            | 5            | chr2           | 346                           | 37608.62 | 7.982 | 8                    |
| CIPIN3 | Cla010532| 564             | 3            | chr2           | 187                           | 19891.53 | 5.463 | 2                    |
| CIPIN4 | Cla012098| 1848            | 6            | chr4           | 615                           | 65610.47 | 7.899 | 8                    |
| CIPIN5 | Cla018455| 1860            | 6            | chr4           | 619                           | 67586.08 | 9.088 | 9                    |
| CIPIN6 | Cla018871| 1896            | 6            | chr6           | 631                           | 69221.59 | 9.178 | 5                    |
| CIPIN7 | Cla018924| 1824            | 7            | chr6           | 607                           | 66418.92 | 9.149 | 9                    |
| CIPIN8 | Cla011709| 1005            | 5            | chr7           | 334                           | 36418.55 | 10.852 | 2                   |
| CIPIN9 | Cla011708| 675             | 1            | chr7           | 224                           | 25017.54 | 7.190 | 5                    |
| CIPIN10| Cla015026| 1449            | 5            | chr9           | 482                           | 53345.51 | 9.198 | 6                    |
| CIPIN11| Cla017028| 1092            | 6            | chr10          | 363                           | 40031.79 | 9.761 | 7                    |
| CIABCB1| Cla009733| 3765            | 12           | chr1           | 1254                          | 135542.15 | 7.431 | 10                   |
| CIABCB2| Cla006778| 3675            | 10           | chr2           | 1224                          | 134618.81 | 7.096 | 11                   |
| CIABCB3| Cla006779| 3708            | 10           | chr2           | 1235                          | 135741.22 | 7.262 | 11                   |
| CIABCB4| Cla010534| 3897            | 12           | chr2           | 1298                          | 139885.34 | 8.658 | 10                   |
| CIABCB5| Cla011266| 3504            | 9            | chr3           | 1167                          | 127972.37 | 9.286 | 8                    |
| CIABCB6| Cla001708| 3750            | 7            | chr5           | 1249                          | 137870.07 | 8.638 | 12                   |
| CIABCB7| Cla007439| 3906            | 12           | chr5           | 1301                          | 141742.47 | 7.289 | 11                   |
| CIABCB8| Cla010011| 3780            | 7            | chr5           | 1259                          | 137952.57 | 8.756 | 9                    |
| CIABCB9| Cla015527| 4368            | 9            | chr9           | 1455                          | 159168.71 | 8.788 | 12                   |
| CIABCB10| Cla016230| 3612            | 12           | chr9           | 1203                          | 131803.14 | 8.908 | 10                   |
| CIABCB11| Cla010337| 3753            | 10           | chr9           | 1250                          | 136474.22 | 8.189 | 10                   |
| CIABCB12| Cla010365| 3750            | 7            | chr9           | 1249                          | 135971.07 | 8.733 | 9                    |
| CIABCB13| Cla004699| 4200            | 11           | chr9           | 1399                          | 155709.89 | 6.722 | 12                   |
| CIABCB14| Cla017800| 4080            | 9            | chr10          | 1359                          | 148875.01 | 6.949 | 11                   |
| CIABCB15| Cla022922| 3633            | 11           | chr11          | 1210                          | 130903.31 | 8.198 | 8                    |
Gene structure analysis of the ClLAX, ClPIN and ClABCB family genes was revealed by comparing the coding sequences with genomic DNA sequences. The exon–intron structures of the three family genes revealed variations (Fig. 3b). The gene exon number of ClLAX genes was either seven or eight. The number of exons in ClPIN genes varied from one (ClPIN9) to seven (ClPIN7). The number of exons in ClABCB genes varied from 7 to 12.

Expression profiles of ClLAX, ClPIN and ClABCB family genes upon IAA treatment
Auxin regulating plant growth and development depends mainly on auxin transporter to regulate auxin relocation and homeostasis [23, 24]. Exogenous auxin treatment could accelerate or block the endogenous auxin transport between different tissues [23, 57]. To investigate whether the auxin transporters in watermelon were regulated by auxin, the expression of ClLAX, ClPIN and ClABCB genes under 10 μM IAA for 9 h in the shoots and roots were analyzed by qRT-PCR (Fig. 4). Total RNA was isolated from the shoots and roots of mock seedlings or IAA-treated seedlings at different time points (6, 12 and 24 h). Our data suggested that most ClLAX, ClPIN and ClABCB genes were auxin responsive genes. The majority of these genes were differentially regulated by IAA at the transcriptional level. IAA treatment increased the expression levels of ClLAX1, −7, ClPIN3, −4, −5, −6, −7 and ClABCB4 in the shoots more than five-fold. On the contrary, ClABCB1, −2, −5, −10 and −14 expression levels were downregulated in the shoots after IAA treatment (Fig. 4a). Most of the auxin transporter genes were upregulated after IAA treatment in the roots (Fig. 4b). IAA treatment upregulated the expression levels of ClLAX1, −2, −3, ClPIN3, −7, ClABCB2, −10 and −12 more than 15-fold in the roots. In both the roots and shoots, the expression of ClABCB5 was down-regulated by IAA treatment.

Expression Profiles of ClLAX, ClPIN and ClABCB family genes under abiotic stresses
Watermelon is one of the most drought and salinity sensitive cucurbit crops. Its yield is significantly influenced by these abiotic stresses such as drought, salinity and cold [52]. Many studies showed that auxin is involved in stress response, and a quantity of auxin transporter genes are associated with abiotic stress responses. To investigate whether ClLAX, ClPIN and ClABCB genes are involved in abiotic stress response, the expressions levels of 33 auxin transporter genes were investigated under salinity (NaCl), drought (PEG) and cold (4 °C) treatment using qRT-PCR (Figs. 5, 6 and 7). Untreated seedlings growing under normal condition were used as control.

![Fig. 1 Chromosomal distributions and expansion patterns of ClLAX, ClPIN and ClABCB family genes in watermelon.](image-url)
Different CILAX, CIPIN and CIABCB expression patterns were observed in the roots and shoots when they were treated with the abiotic stress treatment. The majority of the CILAX, CIPIN and CIABCB genes were downregulated in the shoots after 200 μM NaCl treatment (Fig. 5a). However, most of the CILAX, CIPIN and CIABCB genes were upregulated in the roots after NaCl treatment (Fig. 5b). Only the expression of CILAX6, CIPIN2, and CIABCB5 was inhibited by NaCl treatment in the roots. Half of CILAX, CIPIN and CIABCB genes were upregulated in the roots after PEG treatment (Fig. 6b). Only the expression of CILAX6, CIPIN4, −5, −7, CIABCB7, −8, −9, −11 and −13 were down-regulated in the roots by cold treatment (Fig. 7b).

Expression profiles of CILAX, CIPIN and CIABCB family genes in grafting response

Grafting is an ancient technique that is widely used in agriculture practices to improve productivity and stress resistance [53]. Auxin can increase the activity of cell division and wound healing in cut Arabidopsis inflorescence stems. However, the molecular mechanisms of auxin involved in these processes remain largely unclear. To investigate whether auxin transporter genes from watermelon are involved in grafting response, we analysed the expression profiles of CILAX, CIPIN and CIABCB during grafting for 5 days in the shoots (Fig. 8).
The data indicated that most of the \textit{CILAX} genes were downregulated and most of the \textit{ClPIN} genes were upregulated in the shoots during grafting. Only \textit{ClABCB1}, \textit{−7}, \textit{−11} and \textit{−4} were down-regulated. The rest of the \textit{ClABCB} family genes were significantly upregulated.

**Discussion**

Auxin, as a key regulator of plant growth and development through polar auxin transport, is involved in response to environmental stress [2, 3]. In recent years, the molecular mechanism of auxin transport has been gradually elucidated in \textit{Arabidopsis}. On the basis of the function in auxin transport, auxin transport proteins are divided into three major families. They were AUX/\textit{LAX} influx carriers, PIN efflux carriers and ABCB efflux conditional transporters. With the publication of the \textit{Clanatus} genome [58], we have a further understanding of the molecular mechanism of auxin transport in watermelon. In the current research, we identified 33 auxin transporter genes in watermelon and concentrated on the expression profiles of \textit{ClLAX}, \textit{ClPIN} and \textit{ClABCB} genes to elucidate how the auxin transporters were involved in watermelon responses to salt, drought or cold stresses and the phase of grafting.

**Characterisation of \textit{CILAX}, \textit{ClPIN} and \textit{ClABCB} genes in watermelon**

Watermelon (\textit{Clanatus}), an important vegetable crop with 425 Mb genome size, accounts for approximately 7% of the agricultural area worldwide based on the statistics from Food and Agriculture Organization. Our study characterized the complete \textit{CILAX}, \textit{ClPIN} and \textit{ClABCB} family genes in watermelon. The numbers of \textit{CILAX} and \textit{ClPIN} family genes in watermelon were more than those in \textit{Arabidopsis}. The number of \textit{LAX} genes in watermelon is around twice the number in \textit{Arabidopsis}. The number of \textit{ClABCB} family genes in watermelon is less than that in \textit{Arabidopsis}. \textit{Arabidopsis} homologous genes are widely existed in watermelon genome. The relatively similar protein sequence identities of the LAX, PIN and ABCB proteins between watermelon and \textit{Arabidopsis} implied that all these genes originated from one or more common genes [59]. Two sister pair genes were identified as orthologue genes between watermelon and \textit{Arabidopsis} in PIN family with bootstrap values \(\geq 99\%\). Two sister pair genes were identified in ABCB family between watermelon and \textit{Arabidopsis}. However, no orthologue gene pairs were identified in \textit{LAX} family between watermelon and \textit{Arabidopsis} (bootstrap value \(\geq 99\%\)). \textit{CILAX}, \textit{ClPIN} and \textit{ClABCB} proteins contain multiple transmembrane helices, which are similar to the conserved structure of auxin transport protein from \textit{Arabidopsis} [37, 57]. The \textit{CILAX} proteins only contain one group of transmembrane helices, and there is no variable middle hydrophilic region in \textit{CILAX} proteins (Additional file 4: Figure S1). Two groups of transmembrane helices existed in the N- and C-termini and a highly heterogeneous hydrophilic region was located...
at the centre in most CIPIN and CIABCB proteins (Additional file 4: Figure S1). The PIN protein hydrophilic loop is partially modular for the trafficking behaviour and the intracellular trafficking is plastic depending on cell type and developmental stage [60]. The presence of the hydrophilic region in PIN and ABCB proteins from watermelon suggested that they had a similar trafficking behaviour to Arabidopsis. Phylogenetic and domain structural analyses showed that PIN and ABCB protein functions were conserved between watermelon and Arabidopsis [61].

Tissue-specific expression analysis of CILAX, CIPIN and CIABCB genes

Tissue-specific expression analysis of CILAX, CIPIN and CIABCB genes indicated that the transcriptional level of these auxin transporter genes expressed in the roots, cotyledons, leaves, shoots and flowers varied greatly. LAX, PIN and ABCB genes have been found to be involved in plant growth and development previously [16, 43, 57]. The differential expression level of most of CILAX, CIPIN and CIABCB genes in different tissues showed that they might be involved in the regulation of growth and development in watermelon. In spite of the conservation in protein structure, the CILAX expressed among tissues/organs with different intensities. The high identity of LAX genes between watermelon and Arabidopsis at the protein level indicated that CILAX genes might have conserved function as their Arabidopsis orthologue genes (Additional file 2: Table S2). In Arabidopsis, four AUX/LAX genes have complementary and non-redundant expression profiles in the roots and facilitate distinct developmental process:AtAUX1 functions in root gravitropism [12] and root hair development [62]; AtLAX2 functions in vascular development and cell division in the QC [16, 17]; AtLAX3 and AtAUX1 coordinately regulates apical hook development [63] and lateral root development [13]. The CILAX genes might play similar or different roles during watermelon development because of their variety of expression patterns. PIN family genes have been previously elucidated to participate in growth and development in a variety of plant species [27]. AtPIN1 is expressed during early embryonic development. Later, it expressed in the primary root and in the inflorescence stems [33]. Three
OsPIN5 homologous genes exist in rice genome. OsPIN5a and OsPIN5c weakly expressed in roots, highly expressed in leaves, shoot apex, and panicle. OsPIN5b expressed in young panicles and may be involved in inflorescence formation in rice [37]. ZmPIN1b, an orthologue of AtPIN1, is highly expressed during female inflorescence development in maize [34]. Our data showed that two ClPIN genes (ClPIN3 and ClPIN10) were more highly expressed in the roots than in any other tissues, suggesting that they may function in root development. The subclass B of the ABC superfamily includes the majority of proteins that are able to bind and transport auxin in Arabidopsis. However, other members transport other substrates. The AtABCB14 was first described as a malate transporter [49]. To date, there has been no functional characterization of the ABCBs in watermelon and the likely role of members in auxin transport. We sought to identify candidate CIABCBS with the function of auxin transport. Our phylogenetic analysis showed that the CIABCB11 and CIABCB14 cluster along with AtABCB19 and AtABCB1, respectively, both of which were known as IAA transporters. Further investigation, including cell-type specific expression pattern analysis of these family genes and expression patterns during different developmental processes, is required to reveal how these genes participated in the development regulation functions.

Expression patterns analysis of ClLAX, ClPIN and ClABCB family genes upon IAA treatment
To determine whether the auxin transporters were involved in auxin signal, we analysed the gene expression profiles of these genes at different times under IAA treatment. In Arabidopsis, AtLAX1 and AtLAX3 were highly induced by 2, 4-D in the roots [16]. The expression of AtPIN6 is upregulated by auxin though repressive chromatin modification [64]. The expression level of AtABCB4 is enhanced by 2, 4-D treatment [65] and AtABCB1 is also up-regulated by exogenous auxin application [41]. OsABCB14 was induced rapidly by exogenous auxin in rice. The expression of OsPIN1a showed a five-fold increase after IAA treatment [37]. In maize, most of the auxin transporter genes responded to auxin treatment...
in both shoots and roots [24]. *ClPIN5* and *ClPIN7*, two orthologue genes of *AtPIN1* in watermelon, were also drastically induced by IAA treatment. *ClABCB14* and *ClABCB11*, the orthologue of *AtABCB1* and *AtABCB19* in watermelon, respectively, were up-regulated after IAA treatment in the roots.

**CILAX, ClPIN and ClABCB genes were related to salt, drought, cold and grafting response**

As one of the most important phytohormones, auxin regulates plant growth and mediates various environmental stress responses by controlling several auxin-responsive genes. Recently, evidence has indicated that environmental stresses change auxin distribution and homeostasis mediated by auxin transporters [66, 67]. It has been reported that various abiotic signals can change auxin distribution by modulating the expression of auxin transporter genes [66]. In soybean, abiotic stress and hormonal treatments altered auxin accumulation and distribution in the roots. In addition, under these conditions, some *GmPIN* genes might contribute to auxin distribution and homeostasis [68]. In rice, overexpression of *OsPIN3t* improved drought tolerance and knockdown of *OsPIN3t* led to insensitive to drought stress [36]. Therefore, auxin transporters might mediate the crosstalk between auxin and abiotic stresses. The majority of the *CILAX, ClPIN* and *ClABCB* genes were responsive to cold, drought and high salinity both in the shoot and root tissues. The expression profiling of *CILAX, ClPIN* and *ClABCB* genes changed under abiotic stresses, which might accelerate or decelerate the transportation of endogenous auxin in watermelon seedlings. The responses of auxin transport genes to highly saline and drought stress and their different expression profiles indicated that the transcriptional expressions of these auxin transporter genes were regulated by the different physiological signals. Auxin redistribution and transport may be required for watermelon when it responded to abiotic stresses.

Low-temperature stress is a common adversity, which is often encountered in plant cultivation [69]. Many studies have indicated that a relationship between auxin and low temperature stress [70]. Cold stress changes the

![Fig. 6](image-url)
growth and development of plants closely related to the intracellular concentration gradient of auxin, which is regulated by asymmetric localisation and intracellular trafficking of auxin carriers. For example, the asymmetric redistribution and intracellular cycling of AtPIN3 protein were blocked by cold stress. Cold stress also inhibits the intracellular cycling of AtPIN2 [71]. During low temperature stress, the immobilisation of PINs represents a selective process to regulate the activity of specific proteins, which provides a mechanistic basis to explain the role of auxin in regulating the growth and development of plant under cold stress. Watermelon is
an annual herb of the gourd family, originating from tropical Africa. Most varieties of watermelon are weak to cold hardiness and vulnerable to seasonal restrictions. The transcriptional level of most \textit{CILAX}, \textit{CIPIN} and \textit{CIABCB} genes also changed during the cold treatment, which suggested that these genes may function in the mechanism that helps watermelon tolerate cold stress.

Auxin plays a pivotal role in development, and the mode of auxin flow through a tissue determines the sites of vein formation [72]. Similarly, auxin promotes the formation of the xylem and phloem in callus [73]. In \textit{Arabidopsis}, normal vein development depends on polar auxin transport and can be modified by auxin transport inhibitors or mutations of auxin transport genes [74]. The expression levels of most \textit{CILAX}, \textit{CIPIN} and \textit{CIABCB} genes also changed during grafting. This condition suggested that these genes might play a significant role in auxin transported to graft junction, thereby promoting wound healing and vascular formation.

Some members of auxin transporter family genes have been found to engage in the response to abiotic stresses (such as alkaline, drought, heavy metal, high salinity, nutritional deficiency and cold stress). In \textit{Arabidopsis}, AtAUX1 played an important role in plant tolerance to oxidative stress caused by arsenite [75]. Shoot-supplied ammonium root architecture by interfering with AUX1-dependent auxin transport [76]. Auxin homeostasis is changed in roots under cadmium stress with AUX1-dependent auxin transport [76]. Auxin homeostasis is changed in roots under cadmium stress via AUX1 proteins both in \textit{Arabidopsis} and rice [22, 77]. Aluminium toxicity altered auxin distribution through AtPIN2 and AtAUX1 auxin transporter proteins [78]. AtPIN2 helps roots adapt to alkaline stress by modulating root tip proton secretion [79]. The expression levels of AtPIN3 and AtPIN1 genes were reduced under oxidative stress caused by alloxan [80]. \textit{AtABCB} genes responding to light, CO$_2$, phytochromes and cryptochromes have and root tip proton secretion [79]. The expression levels of \textit{AtPIN2} helps roots adapt to alkaline stress by modulating \textit{AtABCB} family genes in five tissues. [DOX 1910 kb]

The distinct expressions of \textit{CILAX}, \textit{CIPIN} and \textit{CIABCB} genes indicated different regulatory action of these genes in watermelon tolerance to abiotic stresses.

Conclusions

In summary, we characterized the transcript pattern of \textit{CILAX}, \textit{CIPIN} and \textit{CIABCB} family genes in watermelon under exogenous IAA treatments or adversity stress. The distinct expressions of \textit{CILAX}, \textit{CIPIN} and \textit{CIABCB} genes indicated different regulatory action of these genes in watermelon tolerance to abiotic stresses.

Additional files

Additional file 1: Table S1. List of qRT-PCR primers used in the present study. (DOCX 1082 kb)

Additional file 2: Table S2. Percent Identity Matrix of LAX family between watermelon and Arabidopsis. (DOCX 648 kb)

Additional file 3: Table S3. qRT-PCR values of the \textit{CILAX}, \textit{CIPIN} and \textit{CIABCB} family genes in five tissues. (DOX 1910 kb)

Additional file 4: Figure S1. Transmembrane helices of \textit{CILAX}, \textit{CIPIN} and \textit{CIABCB}. Protein transmembrane topology was alyzed using the TMHMM Server. (DOX 1872 kb)

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Availability of data and materials

All related data are available within the manuscript and its additional files.

Authors’ contributions

Cly prepared the manuscript draft and contributed the experiment. WQD provided the materials. YHZ analysed the genome sequencing data. ZAH revised the manuscript. ZWL contributed to the design and performed the statistical analysis. 3X and CHZ assisted to draft the manuscript. All authors are read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

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