RESEARCH ARTICLE

Genome-Wide Identification and Expression Profiling Analysis of ZmPIN, ZmPILS, ZmLAX and ZmABCB Auxin Transporter Gene Families in Maize (Zea mays L.) under Various Abiotic Stresses

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Abstract

The auxin influx carriers auxin resistant 1/like aux 1 (AUX/LAX), efflux carriers pin-formed (PIN) (together with PIN-like proteins) and efflux/conditional P-glycoprotein (ABCB) are major protein families involved in auxin polar transport. However, how they function in responses to exogenous auxin and abiotic stresses in maize is largely unknown. In this work, the latest updated maize (Zea mays L.) reference genome sequence was used to characterize and analyze the ZmLAX, ZmPIN, ZmPILS and ZmABCB family genes from maize. The results showed that five ZmLAXs, fifteen ZmPINs, nine ZmPILSs and thirty-five ZmABCBs were mapped on all ten maize chromosomes. Highly diversified gene structures, nonconservative transmembrane helices and tissue-specific expression patterns suggested the possibility of function diversification for these genes. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to analyze the expression patterns of ZmPIN, ZmPILS, ZmLAX and ZmABCB genes under drought, salt and cold treatment. The expression levels of most ZmPIN, ZmPILS, ZmLAX and ZmABCB genes were induced in shoots and were reduced in roots by various abiotic stresses (drought, salt and cold stresses). The opposite expression response patterns indicated the dynamic auxin transport between shoots and roots under abiotic stresses. Analysis of the expression patterns of ZmPIN, ZmPILS, ZmLAX and ZmABCB genes under drought, salt and cold treatment may help us to understand the possible roles of maize auxin transporter genes in responses and tolerance to environmental stresses.

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Introduction

Plants are constantly challenged by environmental stresses, such as high salinity, drought and cold. Many adaptive mechanisms at different levels, including molecular, cellular and physiological processes, have developed to help plants survive in adverse environmental conditions [1,2]. Auxin plays a critical role in the temporal coordination of plant tolerance to stress [3–5] and many environmental stress responses rely on the dynamic distribution of auxin within different plant tissues [6]. From its primary synthesis sites, such as apical meristems and developing leaf tips, auxin flows in an irreversible direction down towards the roots through the stem vascular tissues, and auxin transporters are thought to be involved in this process [7,8]. Auxin transport proteins in plants are grouped into three major families: auxin resistant 1/like aux1 (AUX1⁄LAX) influx carriers, pin-formed (PIN) (together with PIN-like) efflux carriers and P-glycoprotein (MDR/PGP/ABCB) efflux/conditional transporters [9,10].

The auxin influx carrier, AUX1, belongs to the amino acid permease family of proton-driven transporters, and functions in uptake of indole-3-acetic acid (IAA) into cells [11,12]. LAX genes, the paralogs of AUX1, maintain auxin distribution pattern against environmental or developmental influences [13]. In Arabidopsis, AtLAX3 is reported to promote the initiation of lateral root primordia by increasing a selection of cell-wall-remodeling enzymes [14], and a wild cherry Prunus avium gene, PaLAX1, accelerates the uptake rate of auxin into cells and changes the distribution of free endogenous auxin [15].

The PIN gene family was first cloned in Arabidopsis and functioned as auxin efflux transporter encoding genes [16]. In dicotyledonous Arabidopsis, a number of PIN genes have been studied in detail. The PIN family genes are involved in various developmental processes, including phototropism (AtPIN1, AtPIN3 and AtPIN7), vascular bundle differentiation (AtPIN1), apical embryonic structure specification (AtPIN7), alkaline-stress responses (AtPIN2), lateral root formation (AtPIN2), root hair growth (AtPIN5) and root pattern establishment (AtPIN4) [17–20]. The PIN family gene expression patterns are currently an important area of research. Previous studies have shown that AtPIN1 and AtPIN4 expression levels are upregulated by a MADS-domain transcription factor [21] and phytohormones, such as brassinosteroid, selectively down-regulate AtPIN4 and AtPIN7 expression [22]. Recently, the ERECTA family genes were found to be essential for AtPIN1 expression during mid-vein formation of future leaf primordia [23]. The expression patterns of ZmPIN1a and ZmPIN1b, two novel putative orthologs of AtPIN1 in maize, have also been analyzed during maize development. ZmPIN5b protein is localized on basal cell membranes and may be involved in vascular tissues differentiation [24]. PIN-LIKE proteins, which have a low (10%-18%) sequence identity with PINs, are the most recently characterized auxin transport family proteins in Arabidopsis and their involvement in auxin transport across the plasma membrane has been well studied in heterologous systems [10,25].

An ATP-binding cassette (ABC) transporter family has been reported to be responsible for auxin polar movement at the cell level [26]. In Arabidopsis, AtABCB1 and AtABCB19 are involved in auxin export and AtABCB4 catalyzes auxin import [27]. AtABCB1-mediated auxin efflux is modulated by two partners: PINOID (PID) and TWISTED DWARF1. The phosphorylation of PID determines the auxin transporter activity and enhances AtABCB1-mediated auxin efflux [28]. Furthermore, AtABCB19 participates in auxin-mediated differential tropic responses by interacting with PIN proteins [29]. An Arabidopsis plasma membrane-localized ABC transporter, ABCB21, has been identified as a facultative auxin importer/exporter and it is regulated by cytoplasmic auxin concentration [30]. A monocot rice gene, OsABCB14, has been reported to have a role in auxin transport and iron homeostasis, and this was the first evidence that an ABCB gene had been shown to be involved in iron uptake [31].
Recently, some auxin transporter family genes have been studied in other species, such as rice, *Sorghum bicolor*, *Populus trichocarpa* and *Prunus avium* [15,32–34]. Some PIN and ABCB family genes have already been identified in maize [24,35], but the underlying mechanism linking the auxin transporter family gene expression levels and abiotic stresses (salt, drought and cold stresses) in maize is largely unknown. Maize (*Zea mays* L.) is an important cereal crop, and is the staple food for many people, worldwide. Under natural conditions, high salinity, drought and cold are the major environmental stresses experienced by maize plants [36,37]. Our work provides comprehensive information on the ZmPIN, ZmPILS, ZmLAX, ZmABCB gene families and investigated the different spatio-temporal expression patterns of these genes under salt, drought and cold stresses. The distinctive spatio-temporal expression patterns of the ZmPIN, ZmPILS, ZmLAX and ZmABCB genes, and their differential responses to salt, drought and cold stresses will become important research areas to increase abiotic stress tolerance in maize.

**Material and Methods**

**Plant Material and stress treatments**

Maize (*Zea mays* L. inbred line B73) seeds (wild-type) were used in this study. B73 seeds were surface sterilized, washed with sterile water, and germinated in petri plates in chamber overnight at 28°C with a photoperiod of 16-h light and 8-h dark and a relative humidity of 60%. Then the seedlings were transferred to nutrient solution (half-strong modified Hoagland), the pH of the nutrient solution was adjusted to 5.6, and nutrient solution was changed every 3 days. 14-day-old seedlings were used for RNA isolation and stress treatment experiments. Then shoots and roots samples of maize seedlings were collected for RNA isolation respectively. For auxin treatment on maize seedlings, 14-day-old seedlings were soaked in nutrient solution with or without (mock treatment) 10μM IAA for 48 hours, then roots and shoots of maize seedlings at different time points were collected for RNA isolation respectively. Experiment was repeated for 5 times with similar results. Stress treatments were performed as follows: in salt stress experiment, the roots of maize seedlings were immersed in nutrient solution containing 150 mM NaCl for 48 hours [37]; in drought treatment, maize seedlings were planted in sand with MS liquid medium for one week, and then not irrigated for 3 days as drought treatment [33]; for cold treatment, seedlings were put into a 4°C growth chamber for 48 hours [38]. Untreated seedlings were used as controls. Student t-test analysis between mock-inoculated plants and stress-inoculated plants was performed to reveal the differential expression patterns of ZmPIN, ZmPILS, ZmLAX and ZmABCB family genes.

**Identification of PIN, PILS, AUX/LAX and ABCB auxin transporter gene families in maize**

The Hidden Markov Model (HMM) profiles of the PIN, PILS, AUX/LAX and ABCB auxin transporter gene families (Pfam 01490: Transmembrane amino acid transporter protein; Pfam PF03547: Membrane transport protein; Pfam 03547: Membrane transport protein; Pfam 00005: ABC transporter; Pfam 00664: ABC transporter transmembrane region) were employed to identify the PIN, PILS, AUX/LAX and ABCB auxin transporter families of maize. Pfam 01490 was used for AUX/LAX family identification; Pfam 03547 was used for PIN family; Pfam PF03547 was used for PILS family; Pfam 00005 and Pfam 00664 were used for ABCB family. These five profiles were used to search the complete proteome of maize available in phytozome (http://www.phytozome.net/). All the obtained sequences were sorted as unique sequences for further
membrane transport protein domain search using InterProScan Sequence Search (http://www.ebi.ac.uk/Tools/pfa/iprscan/).

Phylogenetic tree building, intron/exon structure, genome distribution and motif prediction

Multiple sequence alignments were performed on the PIN, PILS, LAX and ABCB proteins using ClustalW with the default parameters, and the alignments were then adjusted manually. The information of *Arabidopsis* and rice auxin transporter encoding genes were listed in S1 Table. The alignments were visualized subsequently by software GeneDoc (http://www.nrbsc.org/gfx/genedoc/), and a phylogenetic tree was constructed with aligned five ZmLAX protein sequences, fifteen ZmPIN protein sequences, nine ZmPILS protein sequences and thirty-five ZmABCB protein sequences using MEGA5.1 (http://www.megasoftware.net/mega5/mega.html) employing the neighbor-joining (NJ) method. The coding sequences (CDS) were obtained from the maize sequencing database. Exon-intron organizations of ZmPIN, ZmPILS, ZmLAX and ZmABCB genes were identified by comparing the coding sequences with their corresponding genomic sequences using Gene Structure Display Server (GSDS) software (http://gds.cbi.pku.edu.cn/). We drew a map of the distribution of ZmPIN, ZmPILS, ZmLAX and ZmABCB genes throughout the maize genome.

Transmembrane helices structure predictions

The transmembrane domains were estimated using TMHMM2: http://www.cbs.dtu.dk/services/TMHMM/. The data of all ZmLAX, ZmPIN, ZmPILS and ZmABCB proteins were listed in S1 Fig.

Expression analysis of maize auxin transporter genes

Spatial-temporal expression pattern analysis of ZmLAXs, ZmPINs, ZmPILSs and ZmABCBs using microarray data for sixty types of tissues and organs housed in the Bio-Array Resource for Plant Biology at the Maize Genetics and Genomics Database (MaizeGDB: http://www.maizegdb.org/expression/) were used. To confirm the microarray data, we chosen four representational tissues and organs including leaves, roots and shoots from two-week seedlings and flowers from two-month plants to test the expression levels of these auxin transporter genes by qRT-PCR methods. The database contains expression as reported by Sekhon et al 2011 mapped to B73 RefGen_v2 [39]. The methods utilized for normalization and to adjust background, as well as detection calls, P-value calculation and adjustment have been described previously [39]. The raw data from the MaizeGDB about these auxin transporter genes is listed in S2 Table.

RNA isolation and Quantitative RT-PCR

The methods, including RNA extraction from various tissues, reverse transcription and qRT-PCR, were performed according to Shen’s work [33]. Total RNA from different tissues or organs including cotyledons, leaves, roots, shoots and flowers were extracted using a Plant RNeasy Mini kit (Qiagen) according to the manufacturer’s instruction. DNase I was used to remove any genomic DNA contamination from total RNA. The primers sequences of qRT-PCR are listed in S3 Table. In the experiment of tissues-specific expression analysis, the gene *Zm-Actin* and 18S rRNA gene were used as internal standards to calculate relative fold differences basing on the comparative cycle threshold (2^-ΔΔCt) values. The primer sequences of Actin gene were up- CACCTTCTAACGAGCTCC/dn-CAGTCAGGATCTTCATGAGG and the primer sequences of 18S rRNA gene were up-AGTTTGAGGCAATAACAGGTCT /dn-GATGAAATTTCCCAAGATTACC.
Heat map representation was performed using the average Ct value with Treeview 1.6 software to visualize the tissues-specific expression analysis data. The logarithm of expression level compared to ZmACTIN/10000 or 18S rRNA/10000 were used by Treeview to visualize as heat map. The expression levels of ZmACTIN or 18S rRNA genes were defined as log (10000) = 4. Histograms were used to show the data of abiotic stress response experiments. The expression levels of ZmPIN, ZmPILS, ZmLAX and ZmABCB genes under mock treatment were defined as 1. All the expression analysis was carried out for five biological repeats and the values shown in figures represent the average values of these five repeats.

Analysis of auxin and stress-related cis-elements

The promoters (-1500 to -1 bp before ATG) of ZmLAX, ZmPIN, ZmPILS and ZmABCB genes were scanned for auxin and stress-related cis-elements. The sequences data of ZmLAX, ZmPIN, ZmPILS and ZmABCB promoters were obtained from phytozome 10.1. Nine cis-elements were used in this study: dehydration and cold response (DRE/CRT), ABA responsive element (ABRE), ARF1 binding site (AuxRE), SA-responsive promoter element (SARE), environmental signal response (G-box), WRKY binding site (W-box), CAMTA binding site (CG-box), PHR1 binding site (P1BS) and sulfur-responsive element (SURE).

Results

Genome-wide identification of PIN, LAX and ABCB genes in maize

In our study, we used the previously reported PIN, PILS, AUX/LAX and ABCB proteins from Arabidopsis as BLAST queries to search the public genomic database (http://www.phytozome.net/). The hidden Markov model (HMM) profiles (Pfam 01490: transmembrane amino acid transporter protein; Pfam 03547: membrane transport protein; Pfam 00005: ABC transporter; Pfam 00664: ABC transporter transmembrane region) were employed to identify the ZmLAX, ZmPIN, ZmPILS and ZmABCB protein families. A total of five ZmLAX genes, 15 ZmPIN genes, nine ZmPILS genes and 35 ZmABCB genes were identified after comprehensive search. The protein and CDS sequences of these genes were subsequently downloaded. Information on 55 auxin transporter encoding genes, such as: names, locus ID, open reading frame (ORF) lengths, intron/exon numbers, chromosome locations and basic deduced polypeptide parameters, are listed in Table 1.

The sizes of the deduced ZmLAX proteins varied slightly ranging from 485 amino acids (ZmLAX4) to 651 amino acids (ZmLAX5), the corresponding molecular masses varied from 53.87 kDa to 72.48 kDa, and the predicted isoelectric points varied from 8.39 (ZmLAX3) to 10.10 (ZmLAX5). The sizes of the deduced ZmPIN proteins varied evidently ranging from 264 amino acids (ZmPIN5b) to 746 amino acids (ZmPIN15), the corresponding molecular masses varied from 27.64 kDa to 81.26 kDa, and the predicted isoelectric points varied from 6.96 (ZmPIN5b) to 11.79 (ZmPIN14). The sizes of the deduced ZmPILS proteins varied greatly ranging from 435 amino acids (ZmPILS2) to 1731 amino acids (ZmPILS6), the corresponding molecular masses varies from 15.81 kDa to 63.65 kDa, and the predicted isoelectric points varied from 5.83 (ZmPILS1) to 8.85 (ZmPILS9). The sizes of the deduced ZmABCB proteins varied largely ranging from 388 amino acids (ZmABCB23) to 1540 amino acids (ZmABCB7), the corresponding molecular masses varied from 41.81 kDa to 172.91 kDa, and the predicted isoelectric points varied widely from 4.98 (ZmABCB2) to 9.22 (ZmABCB16). The data suggested that different auxin transporter proteins might function in auxin relocation when plants were exposed to different microenvironments.
### Table 1. Auxin transport genes in maize.

| Gene     | Locus ID       | ORF length (bp) | No. of introns | Chr No. | Chr location | Deduced polypeptide |
|----------|----------------|-----------------|----------------|---------|--------------|---------------------|
| ZmLAX1   | GRMZM2G149481  | 1560            | 7              | 3       | 33317543–33321538 | 520               |
| ZmLAX2   | GRMZM2G129413  | 1710            | 2              | 1       | 119652826–119656777 | 570               |
| ZmLAX3   | GRMZM2G127949  | 1470            | 7              | 3       | 178002561–17800959 | 490               |
| ZmLAX4   | GRMZM2G045057  | 1455            | 5              | 4       | 204125069–204127812 | 485               |
| ZmLAX5   | GRMZM2G067022  | 1953            | 6              | 6       | 148967914–148977647 | 651               |
| ZmABC1   | GRMZM2G5820122 | 3123            | 3              | 1       | 9303132–93069111  | 1041              |
| ZmABC2   | GRMZM2G401769  | 2367            | 9              | 1       | 42186629–42192907 | 789               |
| ZmABC3   | GRMZM2G032936  | 1362            | 14             | 1       | 54843018–54851106 | 454               |
| ZmABC4   | GRMZM2G315375  | 4317            | 4              | 1       | 202298103–202305287 | 1379             |
| ZmABC5   | GRMZM2G084181  | 4881            | 27             | 2       | 9114721–9126810  | 1627             |
| ZmABC6   | GRMZM2G072850  | 3798            | 8              | 2       | 6171190–6178089  | 1266             |
| ZmABC7   | GRMZM2G032218  | 1537            | 10             | 2       | 13856499–13864605 | 1540             |
| ZmABC8   | GRMZM2G388539  | 1740            | 8              | 2       | 56923761–56929497 | 580               |
| ZmABC9   | GRMZM2G365957  | 3684            | 5              | 2       | 103336614–103342858 | 1288             |
| ZmABC10  | GRMZM2G167658  | 4551            | 4              | 2       | 109104216–109109170 | 1517             |
| ZmABC11  | GRMZM2G118994  | 3987            | 11             | 3       | 50093167–50099618 | 1329             |
| ZmABC12  | GRMZM2G049351  | 1785            | 5              | 3       | 144080317–144083641 | 595              |
| ZmABC13  | GRMZM2G258560  | 3693            | 8              | 3       | 201622560–201629041 | 1231             |
| ZmABC14  | GRMZM2G086730  | 3879            | 11             | 3       | 227824114–227836467 | 1293             |
| ZmABC15  | GRMZM2G441722  | 3591            | 10             | 4       | 33816544–33821596 | 1197             |
| ZmABC16  | GRMZM2G004748  | 3786            | 9              | 4       | 157609283–157616979 | 1262             |
| ZmABC17  | GRMZM2G146204  | 2418            | 2              | 4       | 234421148–234429271 | 806              |
| ZmABC18  | GRMZM2G072071  | 1944            | 17             | 5       | 6877441–6883253   | 648              |
| ZmABC19  | GRMZM2G843192  | 3768            | 5              | 5       | 172023958–172028243 | 1256             |
| ZmABC20  | GRMZM2G832772  | 3867            | 9              | 7       | 123359731–123389161 | 1289             |
| ZmABC21  | GRMZM2G441722  | 3591            | 10             | 4       | 33816544–33821596 | 1197             |
| ZmABC22  | GRMZM2G082385  | 3807            | 11             | 8       | 64497963–64503645 | 1269             |
| ZmABC23  | GRMZM2G153961  | 1164            | 2              | 8       | 64405469–64406946 | 388              |
| ZmABC24  | GRMZM2G843537  | 2295            | 7              | 8       | 64419486–64423645 | 765              |
| ZmABC25  | GRMZM2G014089  | 3234            | 10             | 8       | 152715030–152719617 | 1078             |
| ZmABC26  | GRMZM2G874756  | 1857            | 1              | 9       | 6998377–7000813   | 618              |
| ZmABC27  | GRMZM2G081573  | 2379            | 21             | 9       | 12828329–12877329 | 793              |
| ZmABC28  | GRMZM2G111903  | 4440            | 12             | 9       | 17739642–17751534 | 1480             |
| ZmABC29  | GRMZM2G113203  | 4431            | 10             | 9       | 57011529–57017762 | 1477             |
| ZmABC30  | GRMZM2G891159  | 4239            | 10             | 9       | 138972581–138980123 | 1413            |
| ZmABC31  | GRMZM2G361256  | 3984            | 9              | 9       | 151361774–151369052 | 1328            |
| ZmABC32  | GRMZM2G333183  | 3720            | 9              | 10      | 80571279–80577409 | 1240             |
| ZmABC33  | GRMZM2G111146  | 3909            | 6              | 10      | 125844982–125849427 | 1303            |
| ZmABC34  | GRMZM2G413774  | 3969            | 10             | 10      | 135364950–135393105 | 1323            |
| ZmABC35  | GRMZM2G085236  | 3798            | 8              | 10      | 144913379–144921010 | 1266            |
| ZmPIN5b  | GRMZM2G148648  | 792             | 4              | 1       | 193455354–193457161 | 264              |
| ZmPIN5c  | GRMZM2G040911  | 1095            | 3              | 2       | 191860487–191864149 | 365              |
| ZmPIN13  | GRMZM2G064941  | 891             | 0              | 2       | 212649453–212650346 | 297              |
| ZmPIN14  | GRMZM2G471745  | 816             | 0              | 2       | 212664065–212664888 | 272              |
| ZmPIN5a  | GRMZM2G025742  | 1146            | /              | 3       | 160753018–160757148 | 382              |

(Continued)
Chromosomal distribution and expansion patterns of ZmPIN, ZmPILS, ZmLAX and ZmABCB genes

Based on the start position of each maize LAX, PIN, PILS and ABCB gene on the chromosomes, we mapped all five ZmLAX genes, 15 ZmPIN genes, nine ZmPILS genes and 35 ZmABCB genes on ten chromosomes unevenly (Fig. 1A, Table 1). Chromosomes 2 and 3 contained the largest number of auxin transporter encoding genes (nine genes in each chromosome), but chromosome 6 only contained one gene. In the monocot, Sorghum bicolor, and the dicot, Arabidopsis, many of the auxin transporter encoding genes were clustered [27,33]. According to the definition of gene clusters [40], only two small gene clusters were identified. The first gene cluster consisted of two ZmPIN genes (ZmPIN13 and ZmPIN14) and the second gene cluster consisted of three ZmABCB genes (ZmABCB22, ZmABCB23 and ZmABCB24) (Fig. 1A).

Gene duplication events, including tandem and segmental duplications, are the main contributors to evolutionary momentum [41,42]. ZmLAX, ZmPIN, ZmPILS and ZmABCB family duplication modes throughout the maize genome were analyzed to uncover the genetic relationships between the different genes. Two segmental duplications occurred in ZmLAX gene family: ZmLAX1/ZmLAX2 and ZmLAX3/ZmLAX5. Interestingly, our data suggested that ZmPIN5a and ZmPIN15 may function as ancestral genes during the evolutionary process. Segmental duplications also occurred between ZmPIN5a and ZmPIN5b/ZmPIN5d, and another two segmental duplications occurred between ZmPIN15 and ZmPIN13/ZmPIN14. There was tandem duplication between ZmPIN13 and ZmPIN14. One segmental duplication occurred in the ZmPILS gene family: ZmPILS7/ZmPILS9, but four segmental duplications were found in the ZmABCB gene family: ZmABCB2/ZmABCB30, ZmABCB9/ZmABCB21, ZmABCB6/ZmABCB35 and ZmABCB7/ZmABCB34 (Fig. 1B).

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\begin{array}{|c|c|c|c|c|c|c|}
\hline
\text{Gene} & \text{Locus ID} & \text{ORF length (bp)} & \text{No. of introns} & \text{Chr No.} & \text{Chr location} & \text{Deduced polypeptide} \\
\hline
\text{ZmPIN9} & \text{GRMZM5G859099} & 1299 & 4 & 3 & 187809760–187812845 & 433 & 46.84 & 7.3 \\
\text{ZmPIN8} & \text{GRMZM5G839411} & 1086 & 4 & 3 & 202930545–202933072 & 362 & 39.94 & 8.87 \\
\text{ZmPIN10a} & \text{GRMZM2G126260} & 2058 & 5 & 3 & 214899506–214904595 & 686 & 73.57 & 9.38 \\
\text{ZmPIN1c} & \text{GRMZM2G149184} & 1791 & 5 & 4 & 182007439–182010555 & 597 & 64.65 & 8.16 \\
\text{ZmPIN1d} & \text{GRMZM2G171702} & 1740 & 5 & 4 & 186642791–186645569 & 580 & 61.05 & 9.11 \\
\text{ZmPIN5d} & \text{GRMZM2G175983} & 828 & 0 & 4 & 163010632–163012173 & 276 & 29.9 & 9.42 \\
\text{ZmPIN15} & \text{GRMZM2G021364} & 2238 & 13 & 5 & 152572571–152581759 & 746 & 81.26 & 9.32 \\
\text{ZmPIN1b} & \text{GRMZM2G074267} & 1785 & 5 & 5 & 206727149–206730565 & 595 & 64.51 & 8.9 \\
\text{ZmPIN1a} & \text{GRMZM2G098643} & 1803 & 5 & 9 & 3650766–3654174 & 601 & 65.19 & 8.77 \\
\text{ZmPIN10b} & \text{GRMZM2G160496} & 1743 & 5 & 9 & 16725140–16727538 & 581 & 61.84 & 8.86 \\
\text{ZmPILS1} & \text{GRMZM2G070563} & 927 & 6 & 2 & 216298100–216300491 & 309 & 34.58 & 5.83 \\
\text{ZmPILS2} & \text{GRMZM2G331322} & 435 & 5 & 2 & 226922493–226924568 & 145 & 15.81 & 6.95 \\
\text{ZmPILS3} & \text{GRMZM2G112598} & 1017 & 5 & 3 & 29620546–29623590 & 339 & 36.8 & 5.9 \\
\text{ZmPILS4} & \text{GRMZM2G050089} & 1299 & 10 & 3 & 185812406–185817191 & 433 & 46.75 & 8.25 \\
\text{ZmPILS5} & \text{GRMZM2G030125} & 1353 & 1 & 4 & 13533953–13542026 & 451 & 49.43 & 6.49 \\
\text{ZmPILS6} & \text{GRMZM2G475148} & 1731 & 7 & 7 & 115242111–115247553 & 577 & 63.65 & 8.81 \\
\text{ZmPILS7} & \text{GRMZM2G043254} & 1362 & 9 & 7 & 133486108–133489711 & 454 & 48.86 & 8.85 \\
\text{ZmPILS8} & \text{GRMZM2G072632} & 1101 & 7 & 10 & 90297677–90299717 & 367 & 40.29 & 7.16 \\
\text{ZmPILS9} & \text{GRMZM2G007481} & 1362 & 9 & 10 & 128473459–128477062 & 454 & 48.89 & 8.85 \\
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\end{array}
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ZmPIN, ZmPILS, ZmLAX and ZmABCB protein structure analysis

Fifteen ZmPIN proteins have been found in maize and most of them have a classical conservative domain structure, which consists of two hydrophobic domains (V1 and V2) and one hydrophilic loop containing three conserved regions (C1-C3) [43]. The ZmPIN14 and ZmPIN5a proteins only contained one hydrophobic loop and lacked the V1 and V2 regions. Most ZmPIN proteins contained nine or ten transmembrane helices. However, ZmPIN14 and ZmPIN5a only had two transmembrane helices, and ZmPIN15 had three (S1 Fig.).

The ZmPILS proteins shared similar conservative domain structure with ZmPIN proteins, and the ZmPILS1 and ZmPILS2 proteins only had two transmembrane helices. All five ZmLAX proteins contained a highly conserved core region, which was composed of ten transmembrane helices. Amino acid composition analysis indicated that C-terminus of the ZmLAX was proline-rich and the N-terminus of ZmLAX was acidic amino acids-rich. Previous studies have shown that the ABCB family was a subgroup of the ABC transporter superfamily [44]. Multiple sequence alignment showed that most ZmABCB proteins contained a universal structure with a nucleotide binding domain and a transmembrane domain. These two domains were separated by a less conserved linker loop. However, some ZmABCB proteins only contained a nonconservative transmembrane domain, such as: ZmABCB3, ZmABCB8, ZmABCB13, ZmABCB17, ZmABCB18, ZmABCB23 and ZmABCB28 (S1 Fig.).
Phylogenetic analysis of the LAX, PIN, PILS and ABCB family genes

A number of studies have revealed the biological functions of the four major auxin transporter encoding gene families in different plant species [30,31,45,46]. Investigation of the evolutionary relationships among maize, Arabidopsis and rice helps us to understand the possible roles in these auxin transporter encoding genes in maize. Some background information on the Arabidopsis and rice auxin transporter genes is listed in S1 Table. Our data showed that the LAX family genes were divided into two subfamilies (I and II). The phylogenetic tree showed that some ortholog genes in maize and rice were more closely related than their equivalents in maize and Arabidopsis. Five ortholog gene pairs existed between the maize and rice LAX gene families: ZmLAX1/OsLAX2, ZmLAX2/OsLAX4, ZmLAX4/OsLAX5, ZmLAX3/OsLAX1 and ZmLAX5/OsLAX3. All the PIN family genes could be classified into three subfamilies (I, II and III), and four PIN ortholog gene pairs were found between maize and rice: ZmPIN1a/OsPIN1a, ZmPIN1d/OsPIN1c/OsPIN1d, ZmPIN10a/OsPIN10a, ZmPIN10b/OsPIN10b, ZmPIN5a/OsPIN5a, ZmPIN8/OsPIN8 and ZmPIN5c/OsPIN5c. One paralog gene pair occurred in the maize PIN family gene: ZmPIN13/ZmPIN15. The PILS proteins were also grouped into three subfamilies (I, II and III). Three PLS ortholog gene pairs have been found between maize and rice: ZmPLS4/OsPLS1, ZmPLS5/OsPLS3 and ZmPLS7/ZmPLS8/OsPLS4. The ABCB genes were grouped into four subfamilies (I, II, III and IV). Twelve ABCB ortholog gene pairs were found between maize and rice: ZmABCB4/OsABCB22, ZmABCB16/OsABCB11, ZmABCB15/OsABCB21, ZmABCB32/OsABCB20, ZmABCB33/OsABCB19, ZmABCB17/OsABCB9, ZmABCB10/OsABCB12, ZmABCB11/OsABCB1, ZmABCB22/OsABCB18, ZmABCB24/OsABCB19 and ZmABCB25/OsABCB5. Totally, four paralog gene pairs existed in maize ABCB gene family: ZmABCB6/ZmABCB35, ZmABCB30/ZmABCB2, ZmABCB9/ZmABCB21 and ZmABCB7/ZmABCB34 (Fig. 2).

Tissue-specific expression pattern and exon–intron structure analysis of ZmPIN, ZmPILS, ZmLAX and ZmABCB

The previously reported the involvement of these auxin transporter encoding genes in the control of the auxin influx and efflux polar transport prompted us to investigate the expression levels of ZmPIN, ZmPILS, ZmLAX and ZmABCB genes in different tissues and organs. The microarray data of 60 types of different tissues and organs housed in the MaizeGDB database were used to analyze the spatial-temporal expression pattern of ZmLAX, ZmPIN, ZmPILS and ZmABCB genes. The results showed that most of the auxin transporter genes in maize had tissue-specific expression patterns (S2 Fig.). To confirm the spatio expression dynamics, qRT-PCR was used to monitor the transcript accumulations of ZmPIN, ZmPILS, ZmLAX and ZmABCB genes in four representational organs. These were the leaves, roots and shoots from 2-week old seedlings and flowers from 2-month old plants.

Most transcripts from the ZmLAX, ZmPIN, ZmPILS and ZmABCB family genes increased in the selected tissues, except for ZmABCB34 (Fig. 3A), whose expression level could not be detected in any of the tissues and organs. A majority of the ZmPIN, ZmPILS, ZmLAX and ZmABCB genes displayed distinct tissue-specific expression patterns across the four tissues and organs. Our data showed that the ZmLAX gene family transcript abundance was highest in the shoots and lowest in flowers, and that ZmPIN1b, ZmPIN5a, ZmPIN10a, ZmPIN13, ZmPIN14 and ZmPIN15 were present in all representative tissues and organs. However, the rest of ZmPIN genes were more weakly expressed in flowers than in the other organs, except for ZmPIN10a and ZmPIN1d, which showed the lowest expression in roots; and ZmPIN5b and ZmPIN8, which showed the lowest expression in leaves. Most of the ZmPIN genes were preferentially expressed in the shoots. In the ZmPILS gene family, ZmPILS8 and ZmPILS9 were much more highly expressed than the other ZmPILS genes. Within a similar way to the ZmPIN
and ZmLAX gene family, ZmABCB gene expression was much higher in the roots, shoots and leaves than in the flowers. The qRT-PCR values of the ZmPIN, ZmPILS, ZmLAX and ZmABCB gene expression levels are listed in S4 Table.

The exon–intron structures of the ZmPIN, ZmPILS, ZmLAX and ZmABCB family genes were highly diversified. The ZmLAX family gene exon numbers varied from three (ZmLAX2) to eight (ZmLAX3). Some ZmPIN family genes had similar gene exon-intron organizations, such as ZmPIN1c, ZmPIN1d, ZmPIN8 and ZmPIN1a, ZmPIN1b, ZmPIN10a. Interestingly, three of the ZmPIN family genes (ZmPIN13, 14 and 5b) only contained one exon and no introns. In the ZmPILS gene family, ZmPILS5 also contained one exon and no introns. The exon-intron organization of the ZmABCB genes varied considerably in terms of intron numbers and intron phase (Fig. 3B). This suggested that the ZmABCB gene structures were shuffled during the evolutionary process [47].

**ZmLAX, ZmPIN, ZmPILS and ZmABCB gene expression regulation by auxin**

Auxin transporters regulate auxin relocation during plant growth and development [48]. Exogenous IAA stimulation accelerates or blocks the endogenous auxin transport between different organs [33,49]. In order to gain a better understanding of how auxin transporter encoding genes are involved in responses to exogenous hormones, we analyzed the expression profiles of ZmLAX, ZmPIN, ZmPILS and ZmABCB genes under 10 μM IAA for 48 hours in the shoots and roots. Total RNA isolated from the shoots and roots of IAA-treated seedlings and control seedlings was subjected to qRT-PCR analysis at different time points (0, 12, 24 and 48 hours).
Our qRT-PCR data indicated that most ZmPIN, ZmPILS, ZmLAX and ZmABCB genes were IAA-responsive genes. The expression levels of these genes under IAA treatment are shown in Fig. 4. The expression change trends for these auxin transporter genes over the 48 hours of IAA treatment were similar. IAA treatment increased ZmPIN5c, ZmPIN14, ZmPIN1c, ZmPIN5d and ZmPIN1a expression levels in the shoots five-fold. In contrast, ZmPIN1d, ZmPIN15, ZmLAX5, ZmABCB16 and ZmABCB27-29 expression levels in the shoots were
sharply reduced by IAA treatment (Fig. 4A). In the roots, IAA treatment up-regulated the ZmPIN5c, ZmPIN1c, ZmPIN1a, ZmPILS3, ZmABCB4 and ZmABCB19 expression levels more than five-fold. However, many genes, such as ZmPIN5b, ZmPIN6d, ZmPIN6e, ZmPILS7 and ZmPILS8; ZmLAX4; ZmABCB6, 7, 9, 10, 12, 13 and 24, were considerably down-regulated in the roots by the IAA treatment (Fig. 4B).

Stress-related cis-elements in the ZmLAX, ZmPIN, ZmPILS and ZmABCB gene promoters

Transcription factor binds to a specific motif that was called cis-element to activate gene transcription in plants [50]. Several cis-elements that are involved in stress responses have been well identified in plants, including dehydration and Cold response (DRE/CRT: RCCGAC) [51], ABA responsive element (ABRE: YACGTGK) [52], ARFs binding site (AuxRE: TGTCTC) [53], SA-responsive promoter element (SARE: TGACG) [54], environmental signal response (G-box: CACGTG) [55], WRKY binding site (W-box: TTGACY) [56], CAMTA binding site (CG-box: VCGCGB) [57], PHR1 binding site (PIBS: GNATATNC) [58] and sulfur-responsive element (SURE: GAGAC) [59]. Here, we scan the ZmPIN, ZmPILS, ZmLAX and ZmABCB gene -1500bp upstream promoter regions with nine stress-related cis-elements to gain clues on how the these gene expressions are responsive to stresses stimuli.

The stress-related motifs were obviously enriched in the promoter regions of ZmPIN, ZmPILS, ZmLAX and ZmABCB genes. The numbers of stress-related cis-elements in the
upstream 1.5 kb regions of ZmPIN, ZmPILS, ZmLAX and ZmABCB family genes were listed in S5 Table.

Expression analysis of ZmLAX, ZmPIN, ZmPILS and ZmABCB genes in response to salt, drought and cold treatment

In order to investigate the potential roles of ZmPIN, ZmPILS, ZmLAX and ZmABCB genes in response to environmental stresses, we analyzed the expression patterns of 64 auxin transporter encoding genes in the shoots and roots under salt (NaCl), drought and cold (4°C) treatment as described in the Materials and Methods section. Untreated seedlings grown in nutrient solution were used as control seedlings.

The roots and shoots produced different ZmLAX, ZmPIN, ZmPILS and ZmABCB expression patterns when they were subjected to the NaCl treatment. Most of the ZmPIN, ZmPILS, ZmLAX and ZmABCB genes were up-regulated in the shoots after 48 hours of NaCl treatment. The only exceptions were ZmPIN5a and ZmPIN10b. However, up-regulation did not generally occur in the roots. Only ZmPIN5c, ZmPIN15, ZmPIN10b and ZmLAX2 were induced by NaCl treatment in the roots. No significant changes in ZmPIN5a, ZmPIN5b, ZmPIN9, ZmPIN1b, ZmLAX5, ZmABCB24-26 and ZmABCB31 expression levels were detected, and the rest genes were significantly down-regulated in the roots. In a similar manner, most of the ZmABCB family genes were also sharply induced (> 100 fold) by NaCl treatment in the shoots, but were significantly down-regulated in the roots (Fig. 5). After a 3-day period of drought treatment, the expression levels of the ZmABCB genes, half of the ZmPIN and ZmLAX genes were up-regulated in shoots. However, the expression levels of most of the ZmPIN, ZmPILS, ZmLAX and ZmABCB genes were down-regulated in the roots (Fig. 6).

Most of the ZmPIN, ZmPILS, ZmLAX and ZmABCB genes were induced in the shoots and reduced in the roots by the 4°C treatment after 48 hours. Specially, only ZmPIN5a expression was largely reduced in the shoots and only ZmPIN15 expression was sharply induced expression in the roots (Fig. 7). The auxin transporter encoding genes were responsive to environmental stresses and the shoots and roots showed opposite expression patterns during salt, drought or cold treatment.

Discussion

Auxin transport plays important roles in maize growth and development, including lateral root initiation and environmental stress resistances [60–62]. Based on their different functions in auxin transport, the auxin transporter encoding genes are divided into four major families. These were ZmPIN, ZmPILS, ZmLAX and ZmABCB [9]. In our study, we totally identified 65 auxin transporter encoding genes in maize and focused on the expression profiles of ZmLAX, ZmPIN, ZmPILS and ZmABCB genes in order to elucidate how the auxin transporter encoding genes were involved in maize responses to abiotic (salt, drought or cold) stresses.

Characterization and analysis of the ZmLAX, ZmPIN, ZmPILS and ZmABCB gene families in maize

Maize (Zea mays L.), a cereal crop with a large genome size (2.3 Gbp), provides food and source of bioethanol worldwide. Our study isolated and characterized the complete ZmLAX, ZmPIN, ZmPILS and ZmABCB family genes in maize [63]. The numbers of LAX, PIN and PILS genes in maize were similar to the numbers of these genes in Arabidopsis, rice and sorghum, but the number of ABCB genes in maize was much higher than that in Arabidopsis, rice and sorghum [33,64]. Genes that were homologous to Arabidopsis or rice LAX, PIN, PILS and ABCB family
 genes were present in the maize genome widely. Monocot gene families are always enlarged due to whole genome duplications, which are assumed to occur in the ancestor of monocots about 70 million years ago [25,65]. The relatively high amino acid identities of the LAX, PIN, PILS and ABCB proteins between maize and the model plants rice and Arabidopsis suggested that all these auxin transporter encoding genes originated from one or several ancestral sequences [66]. As monocots, the phylogenetic relationship between maize and rice is much closer than the phylogenetic relationship between maize and Arabidopsis. Five sister pair genes between maize and rice in the LAX family, four in the PIN family, three in the PILS family and 11 in the ABCB family were identified as ortholog genes with bootstrap values ≥99%. However, no highly conserved ortholog gene pairs (bootstrap value ≥99%) between maize and Arabidopsis were identified (Fig. 2). Interestingly, several monocot-specific PIN, PILS and ABCB genes were present, according to the phylogenetic analysis. The presence of at least one monocot-specific PIN gene has been confirmed by a number of previous studies [24,34,67,68]. The PIN genes from subfamily III were all monocot-specific PINs, which suggests that the monocot PIN family is more divergent than dicot Arabidopsis PIN family [49,67]. The PILS genes that belonged to subfamily III were also monocot-specific genes. Furthermore, the ZmABCB genes that belonged to subfamily IV showed low sequence homology with any AtABCB or OsABCB genes, and may play specific roles in maize growth and development.

**Fig 5.** Expression levels of ZmPIN, ZmPILS, ZmLAX and ZmABCB gene families in response to salt. Expression levels of ZmPIN, ZmPILS, ZmLAX and ZmABCB genes were analyzed by qRT-PCR in both shoot (A) and roots (B) of 14-day-old seedlings, which were treated with 150 mM NaCl (salt) for 48 hours. The relative expression levels were normalized to a value of 1 in mock seedlings. Error bars represent SD from five biological replicates.

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ZmLAX, ZmPIN, ZmPILS and ZmABCB proteins contain several transmembrane helices that are similar to the conserved structure of auxin transporter proteins in Arabidopsis and rice [32,49]. The ZmLAX proteins only have one group of membrane-spanning domains, but no variable middle region (S1 Fig.). Most ZmPIN, ZmPILS and ZmABCB proteins contain two groups of membrane-spanning domains in the N- and C-termini, and a highly heterogeneous hydrophilic region, which is located at the center of each protein (S1 Fig.). The PIN protein hydrophilic loop partially modulates the intracellular auxin homeostasis, which is plastic depending on cell type and developmental stage [69]. The presence of the hydrophilic region in maize PIN and ABCB proteins suggested that they had a similar trafficking behavior to the model plants. A combination of phylogenetic and domain structural analyses showed that PIN and ABCB protein functions were conserved between dicots and monocots [70].

Tissue-specific and auxin response expression pattern analysis of ZmPIN, ZmPILS, ZmLAX and ZmABCB family genes

Tissue-specific expression analysis of ZmPIN, ZmPILS, ZmLAX and ZmABCB genes showed that all these auxin transporter encoding genes were expressed in the leaves, roots and shoots with different intensity. Plant PIN, PILS, LAX and ABCB genes have previously been shown to be involved in growth and development [14,17,27]. The differential expressions of most of the
maize PIN, PILS, LAX and ABCB genes in different tissues and organs indicated that they were actively involved in regulating growth and development in maize. Specially, most ZmPIN, ZmPILS and ZmLAX genes were highly expressed in the shoots, which suggested that these genes were responsible for long-range auxin transport from the shoot tip to the roots. Previous studies have shown that ZmPIN1a and ZmPIN1b genes were highly expressed in lateral root caps and ZmPIN1c was preferentially expressed in the post-embryonic roots and stems [66]. Our data confirmed that ZmPIN1 homologous genes were highly expressed in the roots, which suggested that they may take part in maize root architecture determination [24]. Some ZmPIN genes also had a developmental stage-specific expression pattern. ZmPIN1a and ZmPIN1b were always expressed in the lateral developing primordia of the shoot apical meristem, tassels, ears and in the inner core of the meristems [24,71]. ZmLAX1, a close maize homolog of AtAUX1, showed expression in the tips of primary roots and leaf primordia [72]. However, a further investigation was required to uncover that how these auxin transporter genes were participant in the development regulation under different developmental stages.

Auxin plays important roles in various developmental processes and its transport between different tissues is mediated by influx (AUX/LAX and ABCB) and efflux (PIN, PILS and ABCB) carriers [73]. To determine whether these auxin transporter encoding genes were also involved in phytohormone signaling, we analyzed expression profiles of these auxin transporter gene families under various phytohormone treatments. In Arabidopsis, AtPIN6 is expressed
in specific cell and tissue types, and can be induced by auxin by repressive chromatin modification. [74]. The auxin influx genes, \textit{AtLAX1} and \textit{AtLAX3}, were induced obviously in the roots after auxin treatment [75]. \textit{AtABC4} is up-regulated by 2,4-D application [76] and \textit{AtABC1}, which is located in the shoot and root apices, is also induced by IAA treatment [77]. In rice, \textit{OsABCBI4}, which is involved in auxin transport and iron homeostasis, also rapidly responded to exogenous auxin [31]. In maize, \textit{ZmABCBI35} (GRMZM2G085236), present closest sequence similarity to \textit{OsABCBI4} [70], may be also involved in iron uptake and homeostasis. \textit{OsPIN1a} expression showed a five-fold increase after IAA treatment [32]. Some maize auxin transporter encoding genes were also found to be responsive to auxin stimuli in both the roots and shoots, which is similar to the responses in \textit{Arabidopsis} and rice. The \textit{OsPIN1a} ortholog gene in maize, \textit{ZmPIN1a} and \textit{ZmPIN1c}, were also showed significantly up-regulated expression after IAA treatment (Fig. 4). An ortholog of \textit{AtABCBI} in maize, \textit{Dwarf Brachytic2}, has been reported to be involved in auxin efflux out of meristematic regions in the shoots and roots. Meanwhile, \textit{br2} mutant reduced auxin export out of the shoot apex [70]. The expression of \textit{BR2}, which was renamed \textit{ZmABCBI4} in our study, was induced by IAA treatment in both the roots and shoots. These results suggested that maize auxin transporter genes may be regulated by an auxin feedback mechanism.

\textit{ZmLAX}, \textit{ZmPIN}, \textit{ZmPILS} and \textit{ZmABCBI} genes were involved in salt, drought and cold stress responses

Auxin mediates various abiotic stress responses in plants by controlling a large number of auxin-responsive genes that are thought to be involved in abiotic stress responses [78]. It has been reported that various environmental and abiotic signals can change auxin distribution by modulating trafficking and PIN protein polarities [79]. In our study, the expressions of most \textit{ZmPIN}, \textit{ZmPILS}, \textit{ZmLAX} and \textit{ZmABCBI} genes were up-regulated by high salinity and drought in the shoots, but were down-regulated in the roots. The involvement of these genes in the salt and drought stress responses and their similar expression patterns suggested that the expressions of these auxin transporter encoding genes were regulated by the same physiological signal. High salinity and drought are the major causes of the changes in osmotic pressure in plant cells [80], so it is possible that these \textit{ZmPIN}, \textit{ZmPILS}, \textit{ZmLAX} and \textit{ZmABCBI} genes may regulate maize responses to osmotic stress [81].

Cold stress is one of the major limiting factors on crop growth and productivity [80], and many studies have shown that there is a relationship between auxin and cold stress [82,83]. Cold stress affected plant growth and development regulation is closely linked to the intracellular auxin gradient, which is controlled by polar localization and the intracellular trafficking of auxin transporters [84]. For example, the asymmetric \textit{AtPIN3} and \textit{AtPIN2} protein redistribution and intracellular cycling were blocked by cold stress [85]. The immobilization of PINs during cold stress provides a mechanistic basis to explain the role auxin plays in regulating plant growth and development under low temperature stress [84]. The expression levels of most \textit{ZmPIN}, \textit{ZmPILS}, \textit{ZmLAX} and \textit{ZmABCBI} genes were also changed by cold treatment, which suggested that these auxin transporter encoding genes may function in the mechanism that helps maize tolerate cold stress. Promoter \textit{cis}-elements analysis showed that several stress-related motifs were present in the \textit{ZmPIN}, \textit{ZmPILS}, \textit{ZmLAX} and \textit{ZmABCBI} gene promoter regions (S5 Table). It may be the genetic basis of stress expression regulation in these auxin transporter genes [86].

Some auxin transporter genes have already been reported to be involved in responses to abiotic stresses (such as high salinity, drought, cold and alkaline stress). In \textit{Arabidopsis}, \textit{AtPIN2} helps roots adapt to alkaline stress by modulating root tip proton secretion [18]. In salt-stressed
plants, more lateral root (LR) primordia were induced during the pre-emergence to the emergence stages than the control plants. However, the stress-induced lateral root emergence and proliferation almost were abrogated in the auxin transporter mutant aux1-7 [87]. The expression of a sorghum gene, SbLAX4, was dramatically reduced under various abiotic stresses [33]. These results provide genetic and physiological evidence that auxin influx carriers are involved in the response to environmental stresses. The ZmPIN, ZmPILS, ZmLAX and ZmABCB gene expression profiling changes may accelerate or decelerate the transportation of endogenous auxin. Auxin redistribution is an essential process for plant to survive in the challenge environments. For example, the AtSOS3 gene controls lateral root developmental plasticity and low salt stress adaptation by regulating auxin redistribution and transport [88]. The opposite expression response patterns highlighted the dynamic auxin transport processes that occur between the shoots and roots. Auxin transport and redistribution may be required for maize is responded to abiotic stresses. Further studies, including the biological function identification and genetic analysis of each maize auxin transporter, will improve our understanding of the relationship between auxin transporters and abiotic stresses.

Conclusions

Auxin has a fundamental role in plant development and its polar transport across cellular membranes is the key process for responses to environmental stimuli. In our study, the auxin transporter coding gene families, ZmLAX, ZmPIN, ZmPILS and ZmABCB, were well identified in maize, and the expression profiles of these genes under exogenous hormone treatments or abiotic stresses were also elucidated. The different expressions of ZmPIN, ZmPILS, ZmLAX and ZmABCB genes suggested different regulatory roles of these genes in maize tolerance to abiotic stresses. The opposite expression patterns of these auxin transporter genes in the shoots and roots under abiotic stress indicated that auxin homeostasis was an important component of the maize responses to environmental stresses.

Supporting Information

S1 Fig. The predicted transmembrane helices of the ZmLAX, ZmPIN, ZmPILS and ZmABCB Proteins. The transmembrane domains were estimated using TMHMM2 (http://www.cbs.dtu.dk/services/TMHMM/), and the red peaks show the predicted transmembrane regions of proteins. (TIF)

S2 Fig. Spatial-temporal expression patterns of auxin transporter gene families based on digital expression data. 1:Germinating Seed 24h; 2:Coleoptile 6DAS GH; 3:Coleoptile 6DAS Primary Root; 4:Stem and SAM (V1); 5:Stem and SAM (V3); 6:Stem and SAM (V4); 7:Shoot tip (V5); 8:First Internode (V5); 9:First Internode (V7); 10:Fourth Internode (V9); 11:Immature Tassel (V13); 12:Meiotic Tassel (V18); 13:Anthers (R1); 14:Whole Seedling (VE); 15:Primary Root (VE); 16:Pooled Leaves (V1); 17:Primary Root (V1); 18:Topmost Leaf (V3); 19:First Leaf (V3); 20:Tip of Stage 2 leaf (V5); 21:Base of Stage 2 leaf (V5); 22:Tip of Stage 2 leaf (V7); 23:Base of Stage 2 leaf (V7); 24:Eleventh Leaf (V9); 25:Eleventh Leaf (V9); 26:Thirteenth Leaf (V9); 27:Immature Leaf (V9); 28:Thirteenth Leaf (VT); 29:Immature Cob (V18); 30:Pre-pollination Cob (R1); 31:Silks (R1); 32:Thirteenth Leaf (R2); 33:Innermost Husk (R1); 34:Innermost Husk (R2); 35:Outer Husk (R2); 36:Embryo 16DAP; 37:Embryo 18DAP; 38:Embryo 20DAP; 39:Embryo 22DAP; 40:Embryo 24DAP; 41:Endosperm 12DAP; 42:Endosperm 14DAP; 43:Endosperm 16DAP; 44:Endosperm 18DAP; 45:Endosperm 20DAP; 46:Endosperm 22DAP; 47: Endosperm 24DAP; 48:Seed 2DAP; 49:Seed 4DAP; 50:Seed 6DAP; 51:Seed 8DAP; 52:Seed
10DAP; 53:Seed 12DAP; 54:Seed 14DAP; 55:Seed 16DAP; 56:Seed 18DAP; 57:Pericarp 18DAP; 58:Seed 20DAP; 59:Seed 22DAP; 60:Seed 24DAP.

(TIF)

S1 Table. PIN, PILS, LAX and ABCB family genes in *Arabidopsis* and rice. a Locus ID of gene in TAIR database (http://www.arabidopsis.org/). b Accession number of corresponding protein existed in UniProt database (http://www.uniprot.org/). c The locus ID of genes in TIGR Rice Genome Annotation Project Database (http://rice.plantbiology.msu.edu/). d Accession number of corresponding protein sequence existed in UniProt database (http://www.uniprot.org/).

(DOCX)

S2 Table. Spatial-temporal expression patterns of auxin transporter gene families based on digital expression data.

(XLSX)

S3 Table. The primer sequences of *ZmLAX*, *ZmPIN*, *ZmPILS* and *ZmABCB* genes.

(DOCX)

S4 Table. The values of the expression levels of all *ZmPIN*, *ZmPILS*, *ZmLAX* and *ZmABCB* genes.

(DOCX)

S5 Table. Number of stress-related cis-elements in the promoter regions of *ZmPIN*, *ZmPILS*, *ZmLAX* and *ZmABCB* genes.

(DOCX)

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Author Contributions

Conceived and designed the experiments: RY ST CS HW. Performed the experiments: RY YY TS JQ SY XH. Analyzed the data: YY TS JQ. Contributed reagents/materials/analysis tools: RY ST LZ XH. Wrote the paper: ST CS.

References

1. Verslues PE, Agarwal M, Katiyar-Agarwal S, Zhu J, Zhu JK (2006) Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. Plant J 45: 523–539. PMID: 16441347

2. Zhao Y, Ma Q, Jin X, Peng X, Liu J, Deng L, et al. (2014) A novel Maize homeodomain-leucine zipper (HD-Zip) I gene, *Zmhdz10*, positively regulates drought and salt tolerance in both rice and Arabidopsis. Plant Cell Physiol 55: 1142–1156. doi:10.1093/pcp/pcu054 PMID: 24817160

3. Zahir ZA, Shah MK, Naveed M, Akhter MJ (2010) Substrate-dependent auxin production by *Rhizobium phaseoli* improves the growth and yield of *Vigna radiata* L. under salt stress conditions. J Microbiol Biotechnol 20: 1288–1294. PMID: 20890099

4. Ha CV, Le DT, Nishiyama R, Watanabe Y, Sulieman S, Tran UT, et al. (2013) The auxin response factor transcription factor family in soybean: genome-wide identification and expression analyses during development and water stress. DNA Res 20: 511–524. doi: 10.1093/dnares/dst027 PMID: 23810914

5. Min L, Li Y, Hu Q, Zhu L, Gao W, Wu Y, et al. (2014) Sugar and auxin signaling pathways respond to high-temperature stress during anther development as revealed by transcript profiling analysis in cotton. Plant Physiol 164: 1293–1308. doi:10.1104/pp.113.223214 PMID: 24481135
6. Tanaka H, Dhonukshe P, Brewer PB, Friml J (2006) Spatiotemporal asymmetric auxin distribution: a means to coordinate plant development. Cell Mol Life Sci 63: 2738–2754. PMID: 17013565

7. Okada K, Ueda J, Komaki MK, Bell CJ, Shimura Y (1991) Requirement of the Auxin Polar Transport System in Early Stages of Arabidopsis Floral Bud Formation. Plant Cell 3: 677–684. PMID: 12324609

8. Rashotte AM, Poupart J, Waddell CS, Muday GK (2003) Transport of the two natural auxins, indole-3-butyric acid and indole-3-acetic acid, in Arabidopsis. Plant Physiol 133: 761–772. PMID: 14526119

9. Titapiwatanakun B, Murphy AS (2009) Post-transcriptional regulation of auxin transport proteins: cellular trafficking, protein phosphorylation, protein maturation, ubiquitination, and membrane composition. J Exp Bot 60: 1093–1107. doi: 10.1093/jxb/ern240 PMID: 18824505

10. Barbez E, Kubes M, Rolcik J, Beziat C, Pencik A, Wang B, et al. (2012) A novel putative auxin carrier family regulates intracellular auxin homeostasis in plants. Nature 485: 119–122. doi: 10.1038/nature11001 PMID: 22504182

11. Young GB, Jack DL, Smith DW, Saier MH Jr. (1999) The amino acid/auxin:proton symport permease family. Biochim Biophys Acta 1415: 306–322. PMID: 9889387

12. Swarup R, Kargul J, Marchant A, Zadik D, Rahman A, Mills R, et al. (2004) Structure-function analysis of the presumptive Arabidopsis auxin permease AUX1. Plant Cell 16: 3069–3083. PMID: 15486104

13. Bainbridge K, Guyomarc'h S, Bayer E, Swarup R, Bennett M, Mandel T, et al. (2008) Auxin influx carriers stabilize phyllotactic patterning. Genes Dev 22: 810–823. doi: 10.1101/gad.462608 PMID: 18347099

14. Swarup K, Benkova E, Swarup R, Casimiro I, Peret B, Yang Y, et al. (2008) The auxin influx carrier LAX3 promotes lateral root emergence. Nat Cell Biol 10: 946–956. doi: 10.1038/ncb1754 PMID: 18623888

15. Hoyerova K, Perry L, Hand P, Lankova M, Kocabek T, May S, et al. (2008) Functional characterization of the presumptive Arabidopsis auxin permease PAAUX1. Plant Physiol 146: 1128–1141. doi: 10.1104/pp.107.109371 PMID: 18184737

16. Petrasek J, Mravec J, Bouchard R, Blakeslee JJ, Abas M, Seifertova D, et al. (2006) PIN proteins perform a rate-limiting function in cellular auxin efflux. Science 312: 914–918. PMID: 16601150

17. Robert HS, Grone P, Stepanova AN, Robles LM, Lokser AS, Alonso JM, et al. (2013) Local auxin sources orient the apical-basal axis in Arabidopsis embryos. Curr Biol 23: 2506–2512. doi: 10.1016/j.cub.2013.09.039 PMID: 24291089

18. Xu W, Jia L, Baluska F, Ding G, Shi W, Ye N, et al. (2012) PIN2 is required for the adaptation of Arabidopsis roots to alkaline stress by modulating proton secretion. J Exp Bot 63: 6105–6114. doi: 10.1093/jxb/ers259 PMID: 23002434

19. Ganguly A, Lee SH, Cho M, Lee OR, Yoo H, Cho HT. (2010) Differential auxin-transporting activities of PIN-FORMED proteins in Arabidopsis root hair cells. Plant Physiol 153: 1046–1061. doi: 10.1104/pp.110.156505 PMID: 20439545

20. Friml J, Benkova E, Bilou I, Wisniewska J, Hamann T, Ljung K, et al. (2002) AtPIN4 mediates sink-driven auxin gradients and root patterning in Arabidopsis. Cell 108: 661–673. PMID: 11893337

21. Garay-Arroyo A, Ortiz-Moreno E, de la Paz Sanchez M, Murphy AS, Garcia-Ponce B, Marsch-Martinez N, et al. (2013) The MADS transcription factor XAL2/AGL14 modulates auxin transport during Arabidopsis root development by regulating PIN expression. EMBO J 32: 2884–2895. doi: 10.1038/embj.2013.216 PMID: 24121311

22. Nakamura A, Goda H, Shimada Y, Yoshida S (2004) Brassinosteroid selectively regulates PIN gene expression in Arabidopsis. Biosci Biotechnol Biochem 68: 952–954. PMID: 15118332

23. Chen MK, Wilson RL, Palme K, Ditengou FA, Shpak ED (2013) ERECTA family genes regulate auxin transport in the shoot apical meristem and forming leaf primordia. Plant Physiol 162: 1978–1991. doi: 10.1104/pp.112.218198 PMID: 23821653

24. Carraro N, Forestan C, Canova S, Traas J, Varotto S (2006) ZmPIN1a and ZmPIN1b encode two novel putative candidates for polar auxin transport and plant architecture determination of maize. Plant Physiol 142: 254–264. PMID: 16944839

25. Balzani S, Johal GS, Carraro N (2014) The role of auxin transporters in monocots development. Front Plant Sci 5: 393. doi: 10.3389/fpls.2014.00393 PMID: 25177324

26. Blakeslee JJ, Bandopaphyay A, Lee OR, Mravec J, Titapiwatanakun B, Sauer M, et al. (2007) Interactions among PIN-FORMED and P-glycoprotein auxin transporters in Arabidopsis. Plant Cell 19: 131–147. PMID: 17237354

27. Geisler M, Murphy AS (2006) The ABC of auxin transport: the role of P-glycoproteins in plant development. FEBS Lett 580: 1094–1102. PMID: 16359667
38. Henrichs S, Wang B, Fukao Y, Zhu J, Charrier L, Bailly A, et al. (2012) Regulation of ABCB1/PGP1-catalysed auxin transport by linker phosphorylation. EMBO J 31: 2965–2980. doi: 10.1038/emboj.2012.120 PMID: 22549467

29. Bandopadhyay A, Blakeslee JJ, Lee OR, Mravec J, Sauer M, Tiliapwatanakun B, et al. (2007) Interactions of PIN and PGP auxin transport mechanisms. Biochem Soc Trans 35: 137–141. PMID: 17233620

30. Kamimoto Y, Terasaka K, Hamamoto M, Takanashi K, Fukuda S, Shitan N, et al. (2012) OsABCB14 functions in auxin transport and iron homeostasis in rice (Oryza sativa L.). Plant J 79: 106–117. doi: 10.1111/tjp.12544 PMID: 24798203

31. Wang JR, Hu H, Wang GH, Li J, Chen JY, Wu P (2009) Expression of PIN genes in rice (Oryza sativa L.): tissue specificity and regulation by hormones. Mol Plant 2: 823–831. doi: 10.1093/mp/ssp023 PMID: 19825657

32. Shen C, Bai Y, Wang S, Zhang S, Wu Y, Chen M, et al. (2010) Expression profile of PIN, AUX/LAX and PGP auxin transporter gene families in Sorghum bicolor under phytohormone and abiotic stress. FEBS J 277: 2954–2969. doi: 10.1111/j.1742-4658.2010.07706.x PMID: 20528920

33. Carraro N, Tisdale-Orr TE, Clouse RM, Knoller AS, Spicer R (2012) Diversification and expression of the PIN, AUX/LAX, and ABCB families of putative auxin transporters in populus. Front Plant Sci 3: 17. doi: 10.3389/fpls.2012.00017 PMID: 22645571

34. Pang K, Li Y, Liu M, Meng Z, Yu Y (2013) Inventory and general analysis of the ATP-binding cassette (ABC) gene superfamily in maize (Zea mays L.). Gene 526: 411–428. doi: 10.1016/j.gene.2013.05.051 PMID: 23747399

35. Xia Z, Liu Q, Wu J, Ding J (2012) ZmRFP1, the putative ortholog of Arabidopsis SDIR1, encodes a RING-H2 E3 ubiquitin ligase and responds to drought stress in an ABA-dependent manner in maize. Gene 495: 146–153. doi: 10.1016/j.gene.2011.12.028 PMID: 22245611

36. Chen J, Xu G, Zheng HQ (2014) Apoplastic barrier development and water transport in Zea mays seedling roots under salt and osmotic stresses. Protoplasma. 252:173–80. doi: 10.1007/s00709-014-0669-1 PMID: 24965373

37. Zhao J, Zhang S, Yang T, Zeng Z, Huang Z, Liu Q, et al. (2014) Global transcriptional profiling of a cold-tolerant rice variety under moderate cold stress reveals different cold stress response mechanisms. Physiol Plant. doi: 10.1111/ppl.12291.

38. Sekhon RS, Lin H, Childs KL, Hansey CN, Buell CR, de Leon N, et al. (2011) Genome-wide atlas of transcription during maize development. Plant J 66: 553–563. doi: 10.1111/j.1365-313X.2011.04527.x PMID: 21299659

39. Holub EB (2001) The arms race is ancient history in Arabidopsis, the wildflower. Nat Rev Genet 2: 516–527. PMID: 11433358

40. Bowers JE, Chapman BA, Rong J, Paterson AH (2003) Unravelling angiosperm genome evolution by phylogenetic analysis of chromosomal duplication events. Nature 422: 433–438. PMID: 12660784

41. Vision TJ, Brown DG, Tanksley SD (2000) The origins of genomic duplications in Arabidopsis. Science 290: 2114–2117. PMID: 11118139

42. Zazimalova E, Krecek P, Skupa P, Hoyerova K, Petrasek J (2007) Polar transport of the plant hormone auxin—the role of PIN-FORMED (PIN) proteins. Cell Mol Life Sci 64: 1621–1637. PMID: 17458499

43. Verrier PJ, Bird D, Burla B, Dassa E, Forestier C, Geisler M, et al. (2008) Plant ABC proteins—a unified nomenclature and updated inventory. Trends Plant Sci 13: 151–159. doi: 10.1016/j.tplants.2008.02.001 PMID: 18299247

44. Ye L, Liu L, Xing A, Kang D (2013) Characterization of a dwarf mutant allele of Arabidopsis MDR-like ABC transporter AtPGP1 gene. Biochem Biophys Res Commun 441: 782–786. doi: 10.1016/j.bbrc.2013.10.136 PMID: 24211579

45. Fazier IC, Vanstaelen M, Simon S, Yin K, Carron-Arthur A, Nisar N, et al. (2013) Role of the Arabidopsis PIN6 auxin transporter in auxin homeostasis and auxin-mediated development. PLoS One 8: e70069. doi: 10.3389/fpls.2012.00017 PMID: 23922907

46. Holub EB, Bartel B (2005) Auxin: regulation, action, and interaction. Ann Bot 95: 707–735. PMID: 15749753

47. Paponov IA, Teale WD, Trebar M, Billou I, Palme K (2005) The PIN auxin efflux facilitators: evolutionary and functional perspectives. Trends Plant Sci 10: 170–177. PMID: 15817418
50. Liu Y, Wang L, Xing X, Sun L, Pan J, Kong X, et al. (2013) ZmLEA3, a multifunctional group 3 LEA protein from maize (Zea mays L.), is involved in biotic and abiotic stresses. Plant Cell Physiol 54: 944–959. doi: 10.1093/pcp/pcr074 PMID: 23543751

51. Sakuma Y, Liu Q, Dubouzet JG, Abe H, Shinozaki K, Yamaguchi-Shinozaki K (2002) DNA-binding specificity of the ERF/AP2 domain of Arabidopsis DREBs, transcription factors involved in dehydration- and cold-inducible gene expression. Biochem Biophys Res Commun 290: 998–1009. PMID: 11798174

52. Osakaite Y, Yamaguchi-Shinozaki K, Shinozaki K, Tran LS (2014) ABA control of plant macroelement membrane transport systems in response to water deficit and high salinity. New Phytol 202: 35–49. doi: 10.1111/nph.12613 PMID: 24283512

53. Ulmasov T, Hagen G, Guilfoyle TJ (1997) ARF1, a transcription factor that binds to auxin response elements. Science 276: 1865–1868. PMID: 9188533

54. Pieterse CM, Van Loon LC (2004) NPR1: the spider in the web of induced resistance signaling pathways. Curr Opin Plant Biol 7: 456–464. PMID: 15231270

55. Williams ME, Foster R, Chua NH (1992) Sequences flanking the hexameric G-box core CACGTG affect specificity of protein binding. Plant Cell 4: 485–496. PMID: 1498606

56. Chen L, Song Y, Li S, Zhang L, Zou C, Yu D (2012) The role of WRKY transcription factors in plant abiotic stresses. Biochim Biophys Acta 1819: 120–128. doi: 10.1016/j.bbadr.2011.09.002 PMID: 21964328

57. Yang T, Poovalah BW (2002) A calmodulin-binding/CGCG box DNA-binding protein family involved in multiple signaling pathways in plants. J Biol Chem 277: 45049–45058. PMID: 12218065

58. Rubio V, Linhares F, Solano R, Martin AC, Iglesias J, Leyva A, et al. (2001) A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. Genes Dev 15: 2122–2133. PMID: 11511543

59. Maruyama-Nakashita A, Nakamura Y, Watanabe-Takahashi A, Inoue E, Yamaya T, Takahashi H (2005) Identification of a novel cis-acting element conferring sulfur deficiency response in Arabidopsis roots. Plant J 42: 305–314. PMID: 15842617

60. Jansen L, Roberts I, De Rycke R, Beeckman T (2012) Phloem-associated auxin response maxima determine radial positioning of lateral roots in maize. Philos Trans R Soc Lond B Biol Sci 367: 1525–1533. doi: 10.1098/rstb.2011.0239 PMID: 22527395

61. Zorb C, Geilfus CM, Muelling KH, Ludwing-Muller J (2013) The influence of salt stress on ABA and auxin concentrations in two maize cultivars differing in salt resistance. J Plant Physiol 170: 220–224. doi: 10.1016/j.jplph.2012.09.012 PMID: 23181973

62. Zhang Y, Paschold A, Marcon C, Liu S, Tai H, Nestler J, et al. (2014) The Aux/IAA4 gene rum1 involved in seminal and lateral root formation controls vascular patterning in maize (Zea mays L.) primary roots. J Exp Bot 65: 4919–30. doi: 10.1093/jxb/eru249 PMID: 24298964

63. Schnable PS, Ware D, Fulton RS, Stein JC, Wei F, Pasmek S, et al. (2009) The B73 maize genome: complexity, diversity, and dynamics. Science 326: 1112–1115. doi: 10.1126/science.1178534 PMID: 19965430

64. Guilfoyle TJ, Hagen G (2007) Auxin response factors. Curr Opin Plant Biol 10: 453–460. PMID: 17900969

65. Paterson AH, Bowers JE, Chapman BA (2004) Ancient polyploidization predating divergence of the cereals, and its consequences for comparative genomics. Proc Natl Acad Sci U S A 101: 9903–9908. PMID: 15161969

66. Forrestan C, Farinati S, Varotto S (2012) The Maize PIN Gene Family of Auxin Transporters. Front Plant Sci 3: 16. doi: 10.3389/fpls.2012.00016 PMID: 22639639

67. Krecek P, Skupa P, Libus J, Naramoto S, Tejos R, Frimal J, et al. (2009) The PIN-FORMED (PIN) protein family of auxin transporters. Genome Biol 10: 249. doi: 10.1186/gb-2009-10-12-249 PMID: 20053306

68. Xu M, Zhu L, Shou H, Wu P (2005) A PIN1 family gene, OsPIN1, involved in auxin-dependent adventitious root emergence and tillering in rice. Plant Cell Physiol 46: 1674–1681. PMID: 16085936

69. Ganguly A, Park M, Kesawat MS, Cho HT (2014) Functional Analysis of the Hydrophilic Loop in Intracellular Trafficking of Arabidopsis PIN-FORMED Proteins. Plant Cell 26: 1570–1585. PMID: 24692422

70. Knoller AS, Blakeslee JJ, Richards EL, Peer WA, Murphy AS (2010) Brachytic2/ZmABCB1 functions in IAA export from intercalary meristems. J Exp Bot 61: 3689–3696. doi: 10.1093/jxb/erq180 PMID: 20581123

71. Forrestan C, Meda S, Varotto S (2010) ZmPIN1-mediated auxin transport is related to cellular differentiation during maize embryogenesis and endosperm development. Plant Physiol 152: 1373–1380. doi: 10.1104/pp.109.150193 PMID: 20944449
72. Hochholdinger F, Wulff D, Reuter K, Park WJ, Feix G (2000) Tissue-specific expression of AUX1 in maize roots. Journal of Plant Physiology 157: 315–319.

73. Mashiguchi K, Tanaka K, Sakai T, Sugawara S, Kawade H, Natsume M, et al. (2011) The main auxin biosynthesis pathway in Arabidopsis. Proc Natl Acad Sci U S A 108: 18512–18517. doi: 10.1073/pnas.1108434108 PMID: 22025724

74. Nisar N, Cuttriss AJ, Pogson BJ, Cazzonelli CI (2014) The promoter of the Arabidopsis PIN6 auxin transporter enabled strong expression in the vasculature of roots, leaves, floral stems and reproductive organs. Plant Signal Behav 9: e27898. PMID: 24487186

75. Peret B, Swarup K, Ferguson A, Seth M, Yang Y, Dhondt S, et al. (2012) AUX/LAX genes encode a family of auxin influx transporters that perform distinct functions during Arabidopsis development. Plant Cell 24: 2874–2885. doi: 10.1105/tpc.112.097766 PMID: 22773749

76. Terasaka K, Blakeslee JJ, Titapiwatanakun B, Peer WA, Bandyopadhyay A, Makam SN, et al. (2005) PGP4, an ATP binding cassette P-glycoprotein, catalyzes auxin transport in Arabidopsis thaliana roots. Plant Cell 17: 2922–2939. PMID: 16243904

77. Geisler M, Blakeslee JJ, Bouchard R, Lee OR, Vincenzetti V, Bandyopadhyay A, et al. (2005) Cellular efflux of auxin catalyzed by the Arabidopsis MDR/PGP transporter AtPGP1. Plant J 44: 179–194. PMID: 16212599

78. Jain M, Khurana JP (2009) Transcript profiling reveals diverse roles of auxin-responsive genes during reproductive development and abiotic stress in rice. FEBS J 276: 3148–3162. doi: 10.1111/j.1742-4658.2009.07033.x PMID: 19490115

79. Friml J (2010) Subcellular trafficking of PIN auxin efflux carriers in auxin transport. Eur J Cell Biol 89: 231–235. doi: 10.1016/j.ejcb.2009.11.003 PMID: 19944476

80. Krep JA, Wu Y, Chang HS, Zhu T, Wang X, Harper JF (2002) Transcriptome changes for Arabidopsis in response to salt, osmotic, and cold stress. Plant Physiol 130: 2129–2141. PMID: 12481097

81. Fujita Y, Yoshiida T, Yamaguchi-Shinozaki K (2013) Pivotal role of the AREB/ABF-SnRK2 pathway in ABRE-mediated transcription in response to osmotic stress in plants. Physiol Plant 147: 15–27. doi: 10.1111/j.1399-3054.2012.01635.x PMID: 22519646

82. Fukaki H, Fujisawa H, Tasaka M (1996) Gravitropic response of inflorescence stems in Arabidopsis thaliana. Plant Physiol 110: 933–943. PMID: 8819870

83. Wyatt SE, Rashotte AM, Shipp MJ, Robertson D, Muday GK (2002) Mutations in the gravity persistence signal loci in Arabidopsis disrupt the perception and/or signal transduction ofgravitropic stimuli. Plant Physiol 130: 1426–1435. PMID: 12428007

84. Rahman A (2013) Auxin: a regulator of cold stress response. Physiol Plant 147: 28–35. doi: 10.1111/j.1399-3054.2012.01617.x PMID: 22435366

85. Shibasaki K, Uemura M, Tsurumi S, Rahman A (2009) Auxin response in Arabidopsis under cold stress: underlying molecular mechanisms. Plant Cell 21: 3823–3838. doi: 10.1105/tpc.109.069906 PMID: 20040541

86. Liu ZB, Ulmasov T, Shi X, Hagen G, Guilfoyle TJ (1994) Soybean GH3 promoter contains multiple auxin-inducible elements. Plant Cell 6: 645–657. PMID: 8038604

87. Zolla G, Heimer YM, Barak S (2010) Mild salinity stimulates a stress-induced morphogenic response in Arabidopsis thaliana roots. J Exp Bot 61: 211–224. doi: 10.1093/jxb/erp290 PMID: 19783849

88. Zhao Y, Wang T, Zhang W, Li X (2011) SOS3 mediates lateral root development under low salt stress through regulation of auxin redistribution and maxima in Arabidopsis. New Phyto 189: 1122–1134. doi: 10.1111/j.1469-8137.2010.03545.x PMID: 21087263