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Comprehensive Analysis and Expression Profiling of PIN, AUX/LAX, and ABCB Auxin Transporter Gene Families in *Solanum tuberosum* under Phytohormone Stimuli and Abiotic Stresses

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Abstract

Background: The phytohormone auxin is known to regulate various aspects of plant growth and development as well as adaptation to environmental stimuli. It is the only plant hormone that exhibits transport polarity that is mediated by three classes of proteins encoded by three families, AUXIN RESISTANT 1/LIKE AUX1 (AUX/LAX) influx carriers, the PIN-FORMED (PIN) efflux carriers, and the ATP-binding cassette B/Multidrug-resistance/P-glycoprotein (ABCB/MDR/PGP) efflux/condition carriers. Extensive studies have been conducted to examine the biological functions of auxin transporter genes using model plants systems and in several plant species. Despite this, information regarding the comprehensive analysis of auxin transporter genes in potato species and information regarding the expression patterns of these genes in response to external stresses remains scarce.

Results: In this study, we conducted a genome-wide annotation of the StLAX, StPIN, and StABCB auxin transporter gene families to examine genomic distributions, gene structures, phylogenic relationships and co-expression analysis. From these analyses, 5 StLAXs, 10 StPINs, and

22 StABCBs were identified in the potato genome, and they were mapped to 12 chromosomes. Constructing co-expression networks based on significance changes in gene expression revealed 18 gene modules and potato auxin transporter genes distributed in ten of them correlating to the development of various tissues. Tissue-specific expression analysis of potato auxin transporter genes indicated that genes from the StLAX family were expressed at significantly higher levels compared to those of the other two gene families, suggesting that the StLAXs may be more important for these designated developmental stages in potato. Quantitative real-time PCR (qRT-PCR) analysis indicated responsiveness of StLAXs, StPINs, and StABCBs to auxin and polar auxin transport inhibitors (PATIs), implying their possible roles in mediating intercellular auxin homoeostasis and redistribution. Additionally, the differential expression levels of the StLAX, StPIN, and StABCB genes under abscisic acid (ABA) and abiotic stresses (salt and drought) were indicative of their specific adaptive mechanisms regulating tolerance to various environmental stimuli. Promoter *cis*-regulatory element analyses were used to explore a large number of auxin-responsive and stress-related *cis*-elements within the promoters of the StLAX, StPIN, and StABCB genes that could account for their responsiveness to diverse stresses.

Conclusions: In summary, we have provided comprehensive information on StLAX, StPIN, and StABCB auxin transporter gene families in potato. The responsiveness of StLAXs, StPINs, and StABCBs to auxin and PATIs that mediated intercellular auxin homoeostasis and redistribution. Additionally, the differential expression levels of StLAX, StPIN, and StABCB genes in response to ABA and abiotic stresses (salt and drought), suggested that these were specific adaptive mechanisms on tolerance to various environmental stimuli. Promoter *cis*-regulatory element description analyses suggested that a number of c*is*-regulatory elements within the promoters of auxin transporter genes in potato targeted by relevant transcription factors to respond to diverse stresses. We are confident that our results provide a foundation for a better understanding of auxin transport in potato, as we have demonstrated the biological

significance of these family genes in hormone signalling and adaption to environmental stresses.

Keywords: potato, auxin transporter, PIN, AUX/LAX, ABCB, abiotic stress

Background

Auxin is a plant hormone that possesses multiple functions that regulate the plant growth and development primarily at the cellular level and in response to diverse environmental stimuli as well [1-5]. Auxin is primarily synthesised in leaf primordium, germinating seeds, root tips, and cambiums. The directional polar auxin transport (PAT) system distributes auxin to the targeted tissues to facilitate apical dominance, embryonic development, vascular tissue development, root meristem maintenance, and organ formation and positioning [6]. The cell-cell polar transport of auxin is mediated by proteins localised in the plasma membrane (PM), and these proteins are members of three distinct gene families, the LAX influx carriers [7], the PIN efflux carriers [8, 9], and the ABCB/MDR/PGP efflux/condition transporters [10-12]. Unique polar transport of auxin forms auxin gradients that result in asymmetric distribution of PIN, AUX/LAX, and ABCB proteins across cells and tissues. Regulation of the uneven distribution of auxin within tissues and organs or throughout the entire plant body via auxin transporters provides an important strategy to execute the auxin functions in controlling various plant developmental processes and in response to stressful environments.

AUX/LAX influx carriers encompass four highly conversed family members, and AUX1 and LAX1-3 are characterized as multi membrane-spanning transmembrane proteins that facilitate the entry of auxin into cells [13]. The founder member AUX1 was initially identified from *Arabidopsis* and primarily functions to promote high-affinity cellular auxin uptake in the root tips [14]. The auxin insensitive1 (*aux1*) mutant exhibits an agravitropic phenotype that is observed in roots after treatment with the auxin influx carrier inhibitor 1-naphthoxyaceticacids (1-NOA), and the gravitropic response in this mutant can be restored by treatment with the membrane-permeable auxin 1-naphthaleneacetic acid

(NAA) [15, 16]. AtLAX2 participates in the vascular development of cotyledons, and disruption of this gene reinforces cell separation in the quiescent centre (QC) and reduces the expression of the auxin response reporter DR5 [13, 17]. LAX3 in combination with AUX1, appears to facilitate lateral root development by targeting the auxin-inducible expression of a selection of cell wall remodelling enzymes. The *lax3* mutation in *Arabidopsis* disrupts lateral root emergence, while the *aux1lax3* double mutant exhibits a reduced number of emerged lateral roots [18]. The AUX/LAX gene family has also been described in different monocotyledons and dicotyledons in response to hormonal and abiotic stress at the transcriptional level [19-23].

The PIN family is the most extensively studied of the auxin efflux carriers among the auxin transporters in plants, and these carriers play an essential role in PAT [24]. The PIN genes were first cloned in *Arabidopsis*, where eight members of this gene family were well characterized [25]. Of these genes, several are involved in controlling diverse developmental processes. AtPIN1 is expressed in the vascular bundle of the root, in the inflorescence stem, and the developing organs [26, 27]. Loss-of-function mutations in this gene result in defective floral organs and the formation of naked, pin-shaped inflorescences, the fused leaves, and other shoot abnormalities [9]. Initially, three identified mutant alleles of PIN2 that possessed a strong root agravitropic phenotype were named independently as *ethylene-insensitive root1 (eir1)*, *agravitropic1 (agr1)*, and *wavy6 (wav6)* [28-30]. AtPIN3 is symmetrically located in the root columella cells; however, this protein rapidly re-localises laterally in response to gravity stimulation [31]. Mutations in the *Arabidopsis* gene PIN3 result in reduced growth and tropic response, and apical hook formation is also altered in *pin3* mutants [31]. The functional involvement of AtPIN4 during organogenesis and embryogenesis has been also demonstrated [25, 27]. Disruption of AtPIN4 affects pattern formation in both the embryo and the seedling roots [32]. Therefore, AtPIN4 plays an essential role in auxin maximization and redistribution within the root tip [32]. AtPIN7 is expressed in the basal lineage in the embryo and then later in the root tips, and this protein displays an

expression pattern that is complementary to that of PIN1 [9]. These loss-of-function phenotypes demonstrate crucial role for these proteins in these developmental processes.

AtPIN5 has been implicated in regulating intracellular auxin homoeostasis and metabolism. *pin5* loss- and gain-of-function mutants have been observed to be defective in root and hypocotyl growth [33]. Endoplasmic reticulum (ER)-localization of PIN6 during auxin homoeostasis is required for nectary auxin response, short stamen development, and auxin distribution during root organogenesis in *Arabidopsis* [34, 35]. Aberrant expression or loss-of-function of PIN6 can interfere with multiple auxin-regulated growth functions, including shoot apical dominance, lateral root primordia development, adventitious root formation, root hair growth, and root waving, indicating that PIN6 acts as a crucial component of auxin homoeostasis [36]. AtPIN8 is preferentially expressed in male gametophytes, with a specific accumulation in pollen from microspore to mature pollen and during pollen germination [37-39]. The effects of loss or gain of function of PIN6, PIN8, and PIN5 are not limited to vein patterning and can extend to the modulation of intracellular auxin response levels [40].

The ATP-binding cassette (ABC) superfamily is a large and diverse group (A-H) of proteins, and over 100 ABC proteins have been identified to date in plants [41]. In the subfamily B (ABCB) that includes homologs of the mammalian MDRs/PGPs, six members of the ABCB transporter family in *Arabidopsis* (AtABCB1, AtABCB4, AtABCB14, AtABCB15, AtABCB19, and AtABCB21) have been reported to mediate cellular auxin transport or auxin derivatives. Of these transporters, AtABCB1, AtABCB4, and AtABCB19 are the best-characterized [42, 43]. Since the first plant MDR-like gene (AtABCB1/PGP1/MDR1) was cloned from *Arabidopsis*, it has been reported that AtPGP1 is localised to the PM and that the corresponding gene is expressed in both the root and shoot apex [44, 45]. Additionally, the loss of AtMDR1 results in epinastic cotyledons and reduced apical dominance. Thus, researchers have speculated that AtPGP1 may transport a growth-regulating molecule known as indole-3-acetic acid (IAA) from the shoot apex to influence the

distribution of the hormone auxin during plant development [46]. AtABCB19 has been identified as a stable PM protein that mainly localises to the vascular tissues of the hypocotyl and to the stelae of the root [42]. Defects in PGP19 impair basipetal auxin transport and result in abnormalities in the straight growth of hypocotyl [47]. Moreover, ABCB19 coordinated with PIN1 functions to direct auxin flow from the shoot apex to a maximum in roots [48]. AtABCB4/PGP4 is a root-specific transporter that is predominantly expressed during early root development, and the expression of this transporter has been observed within the root elongation zone and lateral root, and during root hair initiation. *atpgp4* mutants exhibit several root phenotypes, such as abnormal lateral root initiation, enhanced root hair elongation, and reduced basipetal auxin transport in roots. These phenotypes suggest the direct involvement of AtPGP4 in auxin homoeostasis within the root [49].

Auxin transporter genes have been widely studied throughout the plant kingdom, including monocotyledons such as *Oryza sativa*, *Sorghum bicolor*, and *Zea mays*, and *Arabidopsis thaliana*, *Populus trichocarpa*, *Citrullus lanatus*, *Medicago truncatula* and *Brassica rapa* L.etc belonging to dicotyledons as well [10, 19-23, 50-52]. However, a systematic study of auxin transporter genes in potato is lacking. Given the important roles of auxin transporter proteins during plant growth and development and in response to diverse environmental stimuli, we provide the first comprehensive information detailing the StLAX, StPIN, and StABCB auxin transporter gene families in potato, and we systematically analyse their genomic distributions, gene structures, phylogenic relationships, co-expression analysis and expression profiles. In this study, we emphasise the distinctive spatio-temporal expression patterns of putative StLAX, StPIN, and StABCB genes in response to phytohormone stimuli and abiotic stress. Two PATIs were used to screen for candidate members of auxin transporter gene families responsible for auxin transport. Additionally, *cis*-regulatory element analysis was incorporated to further examine their expression profiling. Our study aims to provide a foundation for the further exploration of the biological functions of auxin transporter genes.

Results and discussion

Genome-wide identification of StLAX, StPIN, and StABCB auxin transporter genes in potato

From the reference genome retrieved from phytozome 12.1.6 (https://phytozome.jgi.doe.gov/pz/portal.html) for *A. thaliana*, the AtAUX/LAX, AtPIN, and AtABCB protein sequences were used as queries to perform the BLAST searches against the available potato protein sequence data (DM_v3.4_pep_nonredundant) of potato downloaded from PGSC. A total of 5 putative StLAXs and 22 StABCBs were identified from the potato genome, and they were named in accordance with their location order on the chromosomes, with the exception that the nomenclature of 10 StPIN genes was set according to sequence similarity to the *A. thaliana* PIN genes based on previous research [53]. Detailed information regarding 37 *S. tuberosum* putative auxin transporter-encoding genes, including gene names, locus IDs, open reading frame (ORF) lengths, exon numbers, chromosome locations, deduced polypeptide basic parameters, transmembrane helices, and subcellular localisation predictions, are provided in **Table 1**.

The deduced StLAX proteins ranged from 468 (StLAX5) to 494 (StLAX1) amino acids in length, and it possessed a molecular weights (MW) ranging from 53.20 kDa (StLAX5) to 55.71 kDa (StLAX1) and isoelectric points (pI) between 7.9 (StLAX4) and 8.8 (StLAX5) (**Table 1**). The prediction for StLAX proteins in regard to their subcellular localisation provides a foundation for further functional research. StLAX1 was predicted to be localised within the cytoplasm, while StLAX2-5 were predicted to be plasma membrane-localized. Furthermore, topology analysis conducted using TMHHM v.2.0 revealed that all of the StLAX proteins possessed 10 transmembrane helices, indicating that their core regions were highly conserved (**Table 1, Figure S1 A**). Notably, membrane protein StLAX1 was predicted to be cytoplasm-localized, which could be supported by the fact that a nearnest paralogue AtLAX2 was unable to be correctly targeted to the PM when ectopically expressed in epidermal cells [13]. This PM targeting defect of AtLAX2 and StLAX1

might be the requirement of them for additional trafficking factors that performed subfunctionalization in other tissues.

The size of the ORF for StPIN proteins varied from 783bp (StPIN8) to 1965bp (StPIN4). The lengths of the corresponding proteins ranged from 260 to 654 amino acids, and they possessed 28.41 kDa to 71.29 kDa molecular masses and predicted pI values of 6.82 to 9.69. The number of transmembrane domains for StPIN proteins ranged from 5 to 9. Deletion of a segmental sequence at the C-terminus of the StPIN3 proteins resulted in the depletion of transmembrane helices (**Table 1, Figure S1 B**). Four StPIN proteins (StPIN2, StPIN5, StPIN6, and StPIN8) were putatively localized within the PM, while StPIN1, StPIN7, and StPIN9 were localized within chloroplasts, and StPIN3 were localized within the cytoplasm. Specifically, StPIN4 was predicted to be localised both in the chloroplasts and the PM, and StPIN10 was predicted to be vacuolar-localized. Various subcellular localisation patterns may be indicative of the specific functions of the PIN gene family.

Table 1. Information on StLAX, StPIN, and StABCB genes and properties of the deduced proteins in potato (*S. tuberosum*).

Gene[a]	Locus ID[a]	ORF length (bp)[a]	No.of extrons	Chromosome Location (bp)[a]	Deducted polypeptid[b]			No. of transmembrane[c]	Subcellular localization[d]
					Length (aa)	MI wt (Da)	pI		
StLAX1	PGSC0003DMT400004027	1485	8	ch01:87287332..87290998 (-)	494	55706.95	8.5797	10	cyto
StLAX2	PGSC0003DMT400021923	1446	7	ch09:210800..214038 (-)	481	54328.39	8.1707	10	plas
StLAX3	PGSC0003DMT400059693	1467	8	ch10:46747590..46751792 (-)	488	54971.25	8.7078	10	plas
StLAX4	PGSC0003DMT400049377	1458	7	ch10:50480526..50485783 (-)	485	54606.6	7.8706	10	plas
StLAX5	PGSC0003DMT400016760	1407	8	ch11:10042232..10046787 (-)	468	53199.12	8.7862	10	plas
StPIN1	PGSC0003DMT400014752	1845	6	ch03:58350702..58354249 (+)	614	67134.08	9.3381	8	chlo
StPIN2	PGSC0003DMT400048251	1896	7	ch07:2647114..2649884 (+)	631	68666.48	9.4981	9	plas
StPIN3	PGSC0003DMT400015267	1395	6	ch04:2170473..2172957 (-)	464	50223.21	6.8243	5	cyto
StPIN4	PGSC0003DMT400078330	1965	6	ch05:4250058..4253070 (-)	654	71290.07	7.3826	9	chlo/plas
StPIN5	PGSC0003DMT400046253	1068	5	ch01:64013966..64017139 (-)	355	39264.82	9.2151	9	plas
StPIN6	PGSC0003DMT400079013	1587	7	ch06:41187368..41193747 (-)	528	57652.42	8.8456	9	plas
StPIN7	PGSC0003DMT400072459	1764	6	ch10:57054506..57057784 (+)	587	63906.07	8.8949	8	chlo
StPIN8	PGSC0003DMT400003569	783	4	ch02:46450539..46452728 (+)	260	28407.25	9.6913	5	plas
StPIN9	PGSC0003DMT400021600	1785	6	ch10:59284672..59287550 (-)	594	64304.33	9.4377	9	chlo
StPIN10	PGSC0003DMT400027309	966	3	ch04:49481160..49482767 (+)	321	35909.45	7.2746	9	vacu
StABCB1	PGSC0003DMT400007960	3789	12	ch02:30568338..30574681 (+)	1262	136961.15	7.3521	9	plas
StABCB2	PGSC0003DMT400003590	3750	10	ch02:46284463..46291628 (+)	1249	136236.51	8.0288	9	plas
StABCB3	PGSC0003DMT400003546	3792	7	ch02:46615100..46621182 (+)	1263	137488.42	8.0619	11	plas
StABCB4	PGSC0003DMT400034908	3780	12	ch03:822878..830598 (+)	1259	136332.61	8.7596	10	plas
StABCB5	PGSC0003DMT400048379	3414	8	ch03:37579500..37585781 (+)	1137	124433.16	8.2246	9	plas
StABCB6	PGSC0003DMT400063067	1917	17	ch03:55025184..55031533 (+)	638	68411.61	8.8478	6	plas
StABCB7	PGSC0003DMT400058977	4584	11	ch03:61365905..61372730 (+)	1527	167888.19	9.0608	12	plas
StABCB8	PGSC0003DMT400018820	3864	12	ch06:336919..342710 (+)	1287	138575.71	7.9275	9	plas
StABCB9	PGSC0003DMT400018812	3639	10	ch06:344414..349662 (+)	1212	130723.67	7.799	8	plas
StABCB10	PGSC0003DMT400027962	3765	7	ch05:11042954..11047763 (-)	1254	137231.31	9.1918	11	plas
StABCB11	PGSC0003DMT400069516	3561	9	ch06:53569963..53576584 (+)	1186	130633.57	7.4101	9	plas
StABCB12	PGSC0003DMT400013988	3681	8	ch07:11605714..11610849 (-)	1226	134428.48	8.6851	11	plas
StABCB13	PGSC0003DMT400049576	3780	7	ch07:54289854..54295427 (+)	1259	137937.76	9.2144	12	plas
StABCB14	PGSC0003DMT400045176	3774	12	ch08:49324870..49334350 (-)	1257	137797.09	8.6611	10	plas
StABCB15	PGSC0003DMT400022893	1920	10	ch09:2609568..2618204 (+)	639	69814.41	8.7886	2	chlo
StABCB16	PGSC0003DMT400009924	4002	10	ch09:5129170..5136543 (+)	1333	145939.27	7.8141	11	plas
StABCB17	PGSC0003DMT400019156	3864	14	ch11:40225813..40235522 (+)	1287	141384.58	9.5827	11	plas
StABCB18	PGSC0003DMT400019085	3885	13	ch11:40240421..40248719 (-)	1294	142465.24	8.3389	12	plas
StABCB19	PGSC0003DMT400074962	2091	17	ch12:13618482..13637049 (-)	696	78066.69	8.8706	4	plas
StABCB20	PGSC0003DMT400030345	3651	7	ch12:52311070..52319362 (+)	1216	133255.89	8.8183	8	plas
StABCB21	PGSC0003DMT400030342	3096	6	ch12:52333684..52340071 (+)	1031	112725.71	8.785	9	plas
StABCB22	PGSC0003DMT400011930	3864	12	ch12:59696990..59702667 (-)	1287	139652.46	7.1317	11	plas

a, gene information was retrieved from the *S. tuberosum* v4.03 genome annotation (phytozome 12.1.6: http://phytozome.jgi.doe.gov/pz/portal.html)

b, protein profiles were calculated using the Pepstats (https://www.ebi.ac.uk/Tools/seqstats/emboss_pepstats/).

c, transmembrane helices were predicted using the TMHHM Server v2.0 (http://www.cbs.dtu.dk/services/TMHMM/).

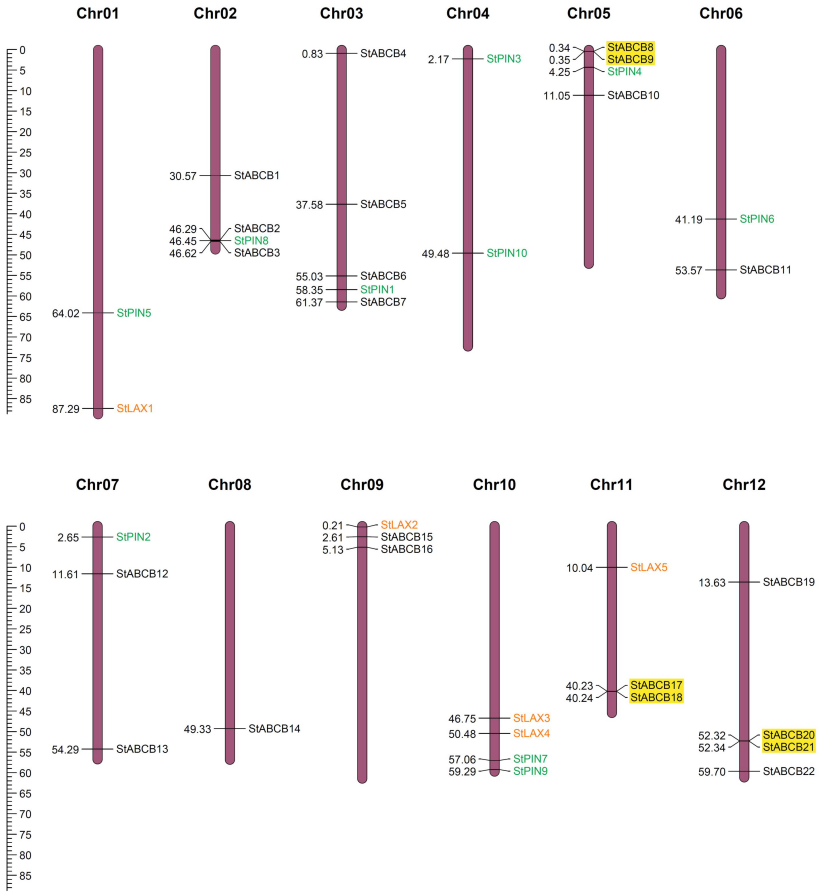
d, subcellular localization was predicted by WoLF PSORT (http://www.genscript.com/psort/wolf_psort.html). Plas, plasma membrane; cyto, cytoplasm；chlo, chloroplast；vacu, vacuolar.

The protein properties of the StABCB family vary dramatically. These proteins ranged from 638 to 1527 amino acids in length, possessed 68.41 kDa to 167.89 kDa molecular masses, and exhibited pI values of 7.35 to 9.58. Additionally, their ORF lengths ranged from 1917bp to 4584bp. For most StABCBs, the conversed domain topology consisted of 2-12 transmembrane helices distributed at the N- and C-termini. The majority of StABCBs possessed a common domain composition with the exception of three members (StABCB6, StABCB15, and StABCB19) that were missing one or both domains (**Table 1, Figure S1 C**). With the exception of StABCB15 that was localised within the chloroplast, all the other StABCBs exhibited a possible localisation to the PM. This implied that these ABCB proteins may act as basal auxin transporters; however, no specialised research has been performed to elaborate on their concrete functions during plant growth and development.

Chromosomal distribution of *S. tuberosum* LAX, PIN, and ABCB auxin transporter gene families

To visualise the organisation of each auxin transporter gene within the *S. tuberosum* genome, chromosomal mapping of 5 StLAXs, 10 StPINs, and 22 StABCBs was arranged based on the position of these genes on 12 different chromosomes (**Table 1, Figure 1**). The 37 genes found to be unevenly distributed, where StLAX1-5 were located in an orderly arrangement on chromosomes 01, 09, 10, and 11, respectively. Chromosomes 01, 09, and 11 contained only one StLAX gene each, while StLAX3 and StLAX4 presented together on chromosome 10. For StPINs, 8 out of the 12 *S. tuberosum* chromosomes contained StPIN proteins, and

no StPIN genes were located on the other four chromosomes (chromosome 08, 09, 11 and 12). Nearly all of chromosomes with the exception of chromosome 01 and 04 contained StABCB genes, and these included four StABCB genes on chromosomes 03 and 12, three StABCBs on chromosomes 02 and 05, and two on chromosomes 07, 09, and 11. Chromosomes 06 and 08 contained only one StABCB gene each. Previous study on potato genome analysis present evidence for at least two whole-genome duplication events [54]. However, analysis of percent ORF nucleotide and amino acid identities of StLAX, StPIN, and StABCB gene families showed no veritable duplicated gene pair shared over 90% identity in both levels (**Table S1, S2, and S3**). Additionally, some of the StABCB genes were clustered and three tandem duplicated ABCB loci pairs (StABCB8-StABCB9, StABCB17-StABCB18, StABCB20-StABCB21) were present on chromosomes 05, 11, and 12, respectively. In contrast, neither the StLAX loci nor the StPIN loci were derived from tandem duplication.



485 **Fig. 1 Chromosomal distribution of StLAX, StPIN, and StABCB family genes.**
486 Potato chromosomes were arranged in blocks. Five StLAX genes, 10 StPIN genes and
487 22 StABCB genes were mapped by locus and the gene clusters were represented by
488 yellow rectangles.

489 **Phylogenetic analysis of LAX, PIN, and ABCB proteins in *Arabidopsis,* rice,**
490 **tomato and potato**

491 To date, auxin transporter-encoding gene families in the model plant
492 *Arabidopsis* have been widely studied for their biological functions in the
493 context of development and responses to the environment [34, 40, 46,
494 55-57]. And there are increasing evidence of roles auxin transporters play
495 in auxin regulated development in monocotyledon and other dicotyledon
496 species [50, 58]. Therefore, investigation of the phylogenetic
497 relationships of auxin transporter proteins from closely related species
498 including *Arabidopsis,* rice, tomato and potato will be helpful for
499 understanding the putative biological functions of auxin transporter genes

in potato. A total of 5 StLAX, 10 StPIN, and 22 StABCB genes, including 4 AtAUX/LAXs, 8 AtPINs, 22 AtABCB proteins (21 transcribed genes and 1 pseudogene) from *Arabidopsis*, 5 OsLAXs, 12 OsPINs, 22 OsABCBs from rice, and 5 SlLAXs, 10 SlPINs, 29 SlABCBs from tomato were uploaded to construct an unrooted neighbour-joining phylogenetic tree (**Figure 2, Table S4**). A phylogenetic tree of the LAX proteins revealed two distinct subfamilies, where potato StLAX proteins clustered more closely with the other dicots (tomato and *Arabidopsis*) compared with those OsLAXs from the monocotyledon species. StLAX2 and StLAX4 proteins were grouped with the closely related SlLAX1 and SlLAX4 proteins, as they possessed 100% amino acid identity (**Figure 2A**). Potato StLAX1 and StLAX3 proteins also shared high homology with *Arabidopsis* AtLAX2. Moreover, a paralogue gene pair was present between potato StLAX5 and *Arabidopsis* AtLAX3 proteins.

A previous study performed a detailed analysis of PIN protein structure, and their findings identified two predicted transmembrane domains linked by the central intracellular loop [59]. More precisely, these possessed long loops were defined as canonical PIN proteins, and truly noncanonical PIN proteins possessed shorter loops. These terms gradually replaced the 'long' and 'short' terms that were previously used to describe the structural features of these proteins. The phylogenetic topology structure of the PIN proteins revealed two major clades. The first, which contained the members with the typical canonical structure, was comprised of *Arabidopsis* AtPIN1 to AtPIN4, and AtPIN7 together with 6 potato PINs (StPIN1-4, StPIN7 and StPIN9), 6 tomato PINs (SlPIN1-4, SlPIN7 and SlPIN9) and four OsPIN1, OsPIN2 and two OsPIN10 proteins. There existed six PIN ortholog gene pairs between potato and tomato, StPIN1-4, StPIN7, StPIN9 and SlPIN1-4, SlPIN7, SlPIN9, respectively. And two paralog gene pairs (StPIN3 with StPIN4 and StPIN7 with StPIN9) existed in the potato PIN gene family. Additionally, four OsPIN1 copies and two OsPIN10 copies showed closer evolutionary relationship, which coincided to the fact that the enlargement of monocot PIN family relied on whole genome duplications and the retention of multiple copies of similar proteins [50] (**Figure 2B**).

The other clade that possessed the noncanonical AtPIN5 was located on the same branch as StPIN5/SlPIN5, StPIN10/SlPIN10, and OsPIN5, and AtPIN8 was grouped with StPIN8 and SlPIN8 due to high sequence similarity. Additionally, PIN6 is unique among the *Arabidopsis* PIN proteins due to its dual localisation at the PM and ER, and based on this, it cannot be classified as canonical PIN protein or a noncanonical PIN protein according to the results of the localisation study. In our study, *Arabidopsis* AtPIN6 and potato StPIN6 proteins constituted a separate clade from the other noncanonical PIN proteins, and this was in accordance with results from a previous publication [35].

A third class of auxin transporters ABCB was reported to mediate almost all aspects of plant growth and development, and several of these proteins (ABCB1, ABCB4, ABCB14, ABCB15, ABCB19 and ABCB21) have been well characterised in regard to their distinct and overlapping functions in *A. thaliana* [43, 56-57, 60-63]. Phylogenetic analysis of the 95 ABCB proteins from *A. thaliana, S. lycopersicum, O. sativa* and *S. tuberosum* genomes indicated that the presence of nine clusters, with potato member(s) in the seven groups (**Figure 2C**). In clade I, AtABCB7 and AtABCB9 were very similar at the sequence level but separate from other ortholog gene pairs (AtABCB3 with AtABCB5, AtABCB11 with AtABCB12 and AtABCB4 with AtABCB21) in *Arabidopsis*. In particular, there was no StABCB protein in potato that was clustered with any AtABCBs, while StABCBs exhibited high sequence similarity to SlABCB proteins from tomato. In contrast to clade I, AtABCB2/10, AtABCB13/14, and AtABCB6/20 were deeply branched in clade VI. Meanwhile, StABCB2 and StABCB16 shared significant homology to ABCB19 and ABCB1, respectively, in *Arabidopsis*, indicating possible roles for StABCB2/16 in auxin transport in plant developmental programs based on close protein sequence similarity to AtABCB19/1. Interestingly, as observed for *Arabidopsis* ABCBs in clade V, five AtABCB genes (AtABCB15-18 and AtABCB22) subclustered with themselves and a number of the OsABCBs grouped in the same way, suggesting that duplication events occurred within these genes.

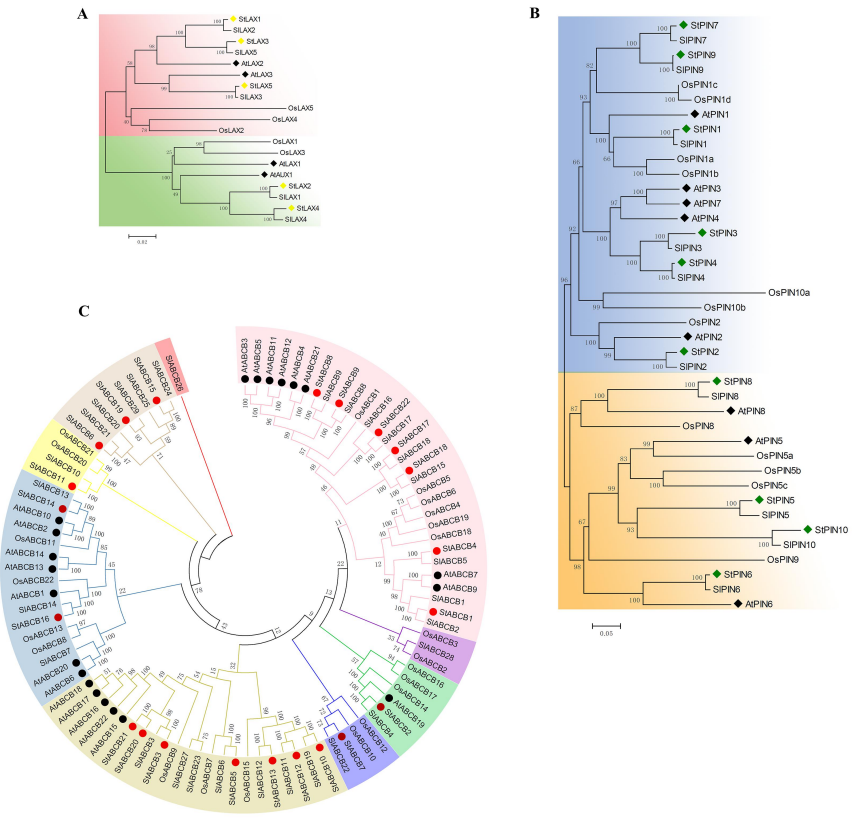


Fig. 2 Phylogenetic tree of LAXs (A), PINs (B), and ABCBs (C) auxin transporter protein families in *Arabidopsis*, rice, tomato and potato. Bootstrap values were presented for all branches. (A) LAX protein family: inventory of AtLAX families was based on TAIR databases. (B) PIN protein family: sequence data on AtPIN and StPIN families was based on TAIR annotation and Efstathios R's publication[53]. (C) ABCB protein family: inventory of AtABCB families was based on the ABC superfamily reviewed by Verrier et al[12]. Different colors indicated different subfamilies.

Weighted co-expression network analysis of *S. tuberosum* genes

A gene expression correlation network was constructed with 25216 differentially expressed genes in various potato tissues by WGCNA method for describing the correlation patterns among genes across microarray samples [64]. A soft thresholding power of 10 with a scale-free model fitting index R^2>0.688 was chosen to maximize scale-free topology and maintaining a high mean connectivity (**Figure 3A and 3B**). Then a dynamic hierarchical tree cut algorithm was constructed to identify stable gene clusters composed of the differentially expressed genes and labelled by unique colors below, including 18 co-expression

584 modules which were named black (983 genes), blue (2705 genes),
585 darkgreen (4172 genes), darkgrey (448 genes), darkmagenta (253 genes),
586 darkred (2526 genes), darkturquoise (461 genes), green (2354 genes),
587 grey60 (530 genes), magenta (2984 genes), paleturquoise (281 genes),
588 pluml (1016 genes), saddlebrown (289 genes), sienna3 (432 genes),
589 skyblue3 (185 genes), tan (1852 genes), turquoise (3445 genes), violet
590 (265 genes), and 35 probesets were added to the "grey" module for they
591 were not grouped in any of the 18 modules (**Figure 3C**). Each module
592 expression profile was summarized by a module eigengene (ME), which
593 assembled the most representative gene expression in a module. To
594 understand the consensus modules significance for biological traits,
595 differential expression of the module corresponding eigengenes across the
596 various potato plant tissues was shown as correlation and *p*-values
597 (**Figure 3D**). Obviously, eigengenes could be characterized by their
598 differential expression profiles in different plant tissues. For example,
599 putative auxin transporter genes in potato investigated in our study were
600 distributed in ten out of 18 consensus modules (**Table S5**). Furthermore,
601 the construction of the cluster dendrogram among module eigengenes
602 resulted in several meta-modules and the relationship between them were
603 highly preserved (**Figure 3E**). For example, one meta-module (comprised
604 of the black and darkgrey module eigengenes) represented by StABCB9
605 and StABCB16 that were differential expressed in leaves. Another
606 meta-module (comprised of the darkgreen and grey60 module eigengenes)
607 represented by StPIN1/2/3 and StABCB3/22 that tended to be
608 differentially expressed in shoot apex. Moreover, the blue and
609 darkmagenta module eigengenes including StPIN5/6, StABCB1/5/6/8/15
610 and StLAX4 tended to be co-expressed in stolon. Thus, this analysis
611 revealed that the meta-modules corresponded to a biologically
612 meaningful characterization of modules and genes.

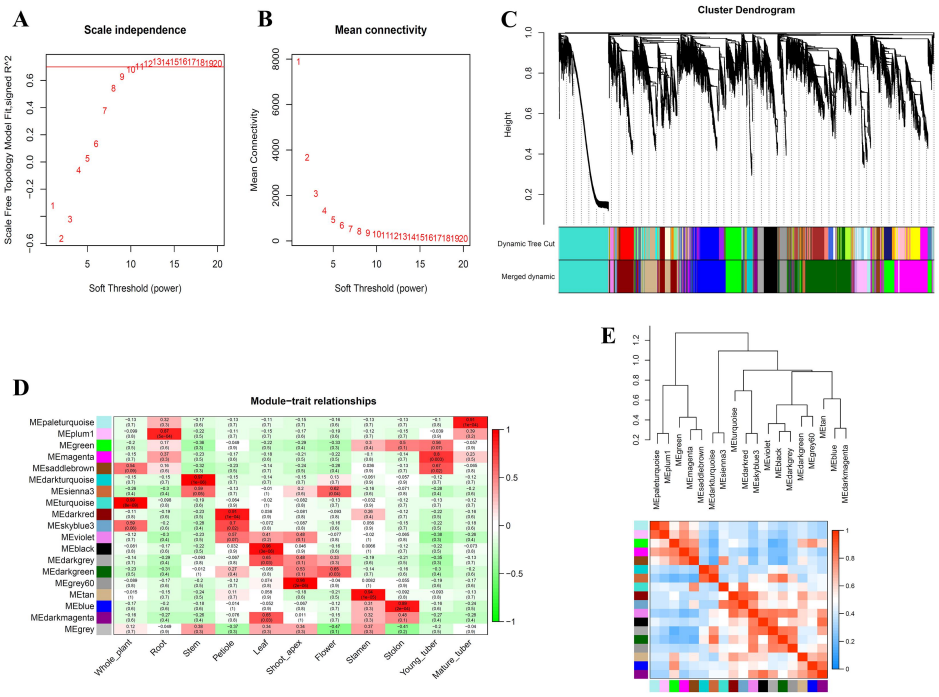


Fig 3. Graphical visualization of the *S. tuberosum* co-expression network. (A) Plot showing the scale free topology R^2 value in function of increasing soft thresholding power. (B) Plot showing the relation between mean connectivity and soft threshold. (C) Dendrograms produced by average linkage of hierarchical clustering of *S. tuberosum* genes, which based on a topological overlap matrix (TOM). The modules were assigned colors as indicated in the horizontal bar beneath the dendrogram. (D) Characterizing consensus modules by differential expression of their corresponding eigengenes in the various tissues from potato plant. Red meant over-expression, green meant under-expression; numbers in each cell gave the corresponding t-test *p*-value. Each column corresponded to a tissue and each row corresponded to an eigengene. (E) Clustering dendrograms of consensus module eigengenes for identifying meta-modules (above) and the heatmap for the correlation coefficient between the modules (below). The diagonal plots showed heatmap plots of eigengene adjacencies. Each row and column corresponded to one eigengene (labeled by consensus module color). Within the heatmap, red indicated high adjacency (positive correlation) and green low adjacency (negative correlation) as shown by the color legend.

Gene structure and tissue-specific expression of StLAX, StPIN, and StABCB family genes

To investigate the similarity and diversity in the gene structures of StLAX, StPIN, and StABCB family genes, exon-intron structure analysis

was performed by comparing the coding sequences with the genomic sequences. The exon number of StLAX genes was either seven or eight, indicating a conversed gene structure (**Figure 4, Table 1**). From the exon-intron organisation of 10 StPIN genes, the structural patterns of seven canonical type PIN genes (StPIN1-4 and StPIN6-7) were highly conserved with 6-7 exons divided by 5-6 introns, while the number of exons in the other three noncanonical StPIN genes (StPIN5, StPIN8 and StPIN10) varied from 3 to 5 and exhibited a smaller gene size in contrast to these canonical StPINs. In the StABCB gene family, the gene annotation predicted 6 to 17 exons, implying a dramatic variation in the exon-intron structure. This variation in both StPINs and StABCBs was primarily due to divergent intron length, which was one of the predominant factors affecting the gene size.

Analysis of the gene expression profiles in different tissues/organs can be helpful for exploring their possible biological functions. Based on available RNA-seq-generated expression data for potato genotype RH, a heat map was constructed to reveal the expression levels of StLAX, StPIN, and StABCB genes in 8 tissues/organs that included flower, leaf, stem, root, stolon, young tuber, mature tuber, and shoot apex (**Figure 4; Table S6**). The results revealed that most transcripts from the StLAX, StPIN, and StABCB family genes were detectable in all selected tissues. Exceptions included a failure to detect StPIN10 and StABCB12 and low detection of StABCB10. In the StLAX gene family, StLAX2 was expressed in all tissues at a high level. The transcript levels of StLAX1 were higher in the stolon and exhibited a tissue-specific expression pattern. The remaining StLAX genes were constitutively expressed in all tissues. Additionally, all StLAX genes exhibited the lowest expression in mature tubers. For StPINs, StPIN1, and StPIN4 were ubiquitously expressed, while 5 out of the remaining StPIN genes were preferentially expressed at a specific developmental stage. StPIN2 and StPIN3 were more highly expressed in young tuber than in other organs, and StPIN5 was abundantly expressed in the roots. Meanwhile, two homologous genes, StPIN7 and StPIN9, exhibited relatively high levels in the stolon tissue, indicating that functional redundancy existed among the PIN genes.

Finally, expression analysis of the StABCB gene family revealed that relatively high expression of StABCB2 and StABCB16 was present in all tissues examined, and that StABCB1, StABCB5, and StABCB6 were expressed in a root-preferential manner, implying that these genes may play specific roles in regulating the development of root organs. A number of genes (including StABCB4, StABCB7, StABCB9, StABCB11, StABCB13, StABCB17, StABCB18, and StABCB20-22) exhibited almost no expression or possessed relatively low levels in all the analysed tissues. To summarize these three auxin transporter gene families, genes of the StLAX family were expressed at significantly higher levels than the other two gene families, suggesting that the StLAXs may be more important for these designated developmental stages in potato.

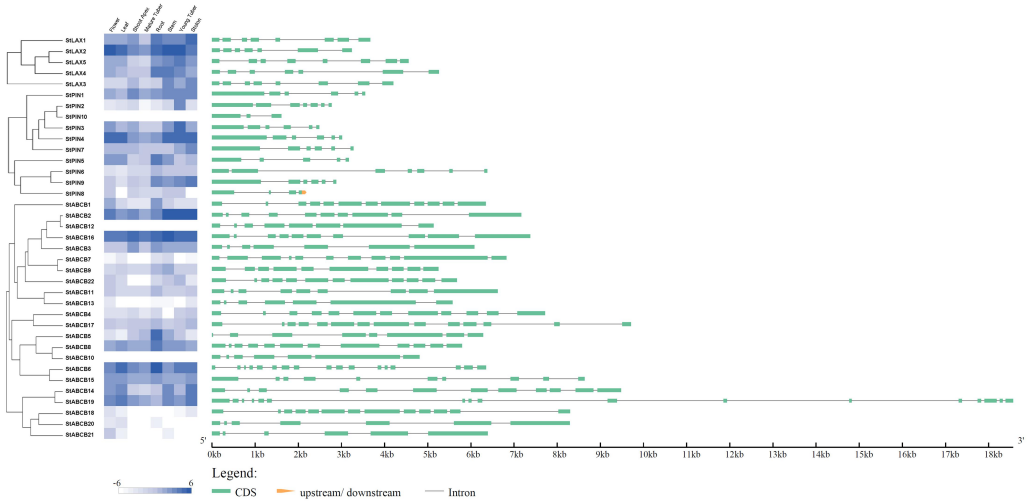


Fig. 4 Tissues-specific expressions and exon-intron structures of StLAX, StPIN, and StABCB genes. The heat map was generated using the Cluster 3.0 software according to the RNA-seq data of RH. The exons were indicated by green boxes and the introns were indicated by gray lines.

Auxin regulation of the StLAX, StPIN, and StABCB genes

Auxin plays critical roles in regulating plant growth and development via influencing auxin transporters and maintaining intracellular auxin homoeostasis and redistribution [65]. Exogenous IAA application may impact auxin stimulation and transport [32]. To determine how auxin transporters in potato respond to exogenous auxin treatment, we tested the expression levels of members of the StLAX,

StPIN, and StABCB gene families in response to treatment with 10μM IAA for 3h using qRT-PCR. The results indicated that most genes were responsive to IAA treatment. In the StLAX family, StLAX1-3 exhibited a lower expression level compared to that of StLAX4, which exhibited a moderate increase. A total of 5 StPIN genes (StPIN1, StPIN2, StPIN5, StPIN7 and StPIN10) were up-regulated, and the remaining StPINs were down-regulated at different levels after IAA treatment. It should be noted that StPIN6 expression in particular strongly decreased. For the StABCB family, the majority of StABCBs exhibited a positive response to auxin, where StABCB1, StABCB3, and StABCB9 sharply upregulated and only 4 genes considerably reduced compared to controls (**Figure 5；Table S7**). Overall, several StLAX, StPIN, and StABCB genes were up-regulated in response to exogenous IAA, suggesting role for these genes in auxin-related biological progress. There has been little functional characterisation of auxin transporters in potato to date. However, phylogenetic relationships with *Arabidopsis* could provide clues in regard to the functional identity of auxin transporter genes in potato [21, 51]. In *Arabidopsis*, the expression of LAX3 was itself auxin-inducible, and LAX3 therefore functioned to promote lateral root emergence, where LAX3-expressing cells became more efficient sinks for auxin [18]. The paralogue of AtLAX3 in potato, StLAX5, exhibited a downwards trend after auxin treatment and may also be involved in root development. It has been reported that StPIN5 was predominantly expressed in the root [53]. Meanwhile, the functional role of the ortholog gene (AtPIN5) found in *A. thaliana* in auxin homoeostasis has been confirmed. Therefore, it is possible that StPIN5 functions to maintain auxin homoeostasis in potato based on the close phylogenetic relationship between AtPIN5 and StPIN5, and current evidence suggested that StPIN5 was up-regulated in shoots and down-regulated in roots in response to auxin treatment [66]. In the ABCB family, AtABCB19 was identified as an IAA transporter with strong induction in response to exogenous auxin treatment[11], while the expression of its presumed ortholog in potato, StABCB2, was not upregulated by IAA, suggesting that there exists a variational relationship between these two proteins. This motivated us to explore the role of

StABCBs in auxin transport. Our findings revealed the existence of a similar feedback mechanism that functioned in regulating the expression of auxin transporter genes in *S. tuberosum*.

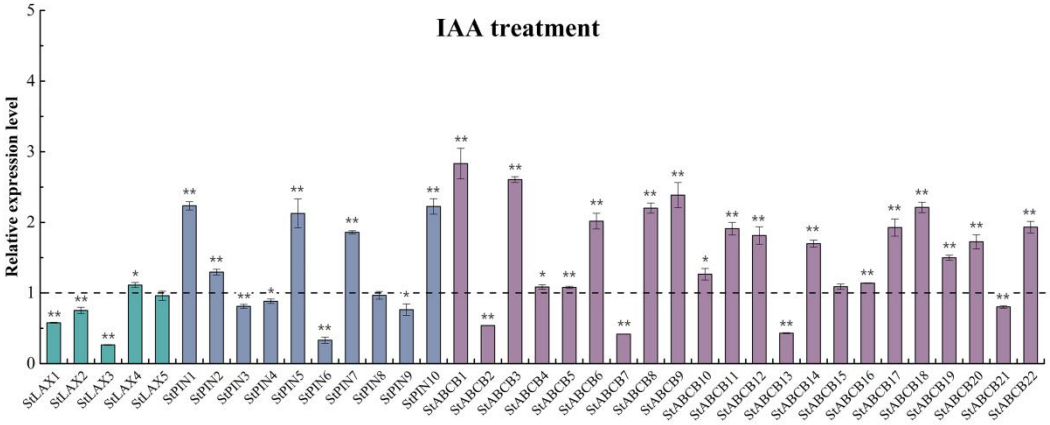


Fig 5. The relative expression values of auxin transporter StLAX, StPIN, and StABCB genes following IAA treatment. Total RNA was extracted from 4-week-old potato plantlets for expression analysis. The histogram represents the relative RNA level of genes after IAA treatment compared with the mock expression level, which was normalized to a value of 1 . The elongation factor 1-a (ef1-a) was employed as the internal standard to normalize the relative mRNA level of individual genes. Error bars represent the standard deviations (SDs) from three biological replicates. (* t-test *P*-value <0.05, **t-test *P*-value <0.01).

Expression analysis of StLAX, StPIN, and StABCB genes in response to PATIs treatments

Directional PAT is a form of active intercellular transport mediated by influx and efflux carriers that controls many important plant growth and developmental processes. It is known that PATIs, including auxin influx carrier inhibitor 1-NOA and two auxin efflux carrier inhibitors NPA (1-Naphthylphthalamic acid) and TIBA (2,3,5-triiodobenzoic acid) are major tools that can be used to explore auxin-dependent biological processes. Here, we conducted a PATI assay to investigate the transcriptional fluctuations of StLAX, StPIN, and StABCB under 1-NOA and TIBA treatments to allow us to gain insights into the effects of PATIs on auxin transporters (**Figure 6a and 6b**；**Table S7**). Our results revealed that most genes were induced by PATIs. Surprisingly, all StLAX genes were inhibited by 1-NOA due to down-regulation. In contrast, most StPIN and StABCB family genes were insensitive to 1-NOA treatment with the

exception of StABCB1, which was up-regulated dramatically in response to treatment with 1-NOA and of StABCB7, StABCB8, StABCB21 and StABCB22 which exhibited a more moderate increase. For TIBA treatment, most StPIN and StABCB auxin efflux carrier family genes were down-regulated or exhibit slight variations, with the exception of StPIN3 and StPIN9, StABCB8 and StABCB22 that exhibited an increased in response to TIBA. However, the response of StLAX genes to TIBA treatment was irregular, where two of 5 StLAXs were up-regulated and three were down-regulated to varying degrees. Overall, the expression of auxin transporter genes may be blocked by the PATIs. Additionally, we excluded the use of an additionally polar auxin transport inhibitor, NPA, in our present study, as a previous publication had pointed out that NPA was invaluable for demonstrating the involvement of the auxin efflux carrier during PAT-mediated developmental processes [67], and TIBA was sensitive to a greater number of genes than was NPA in regard to auxin response genes in *Arabidopsis* [68].

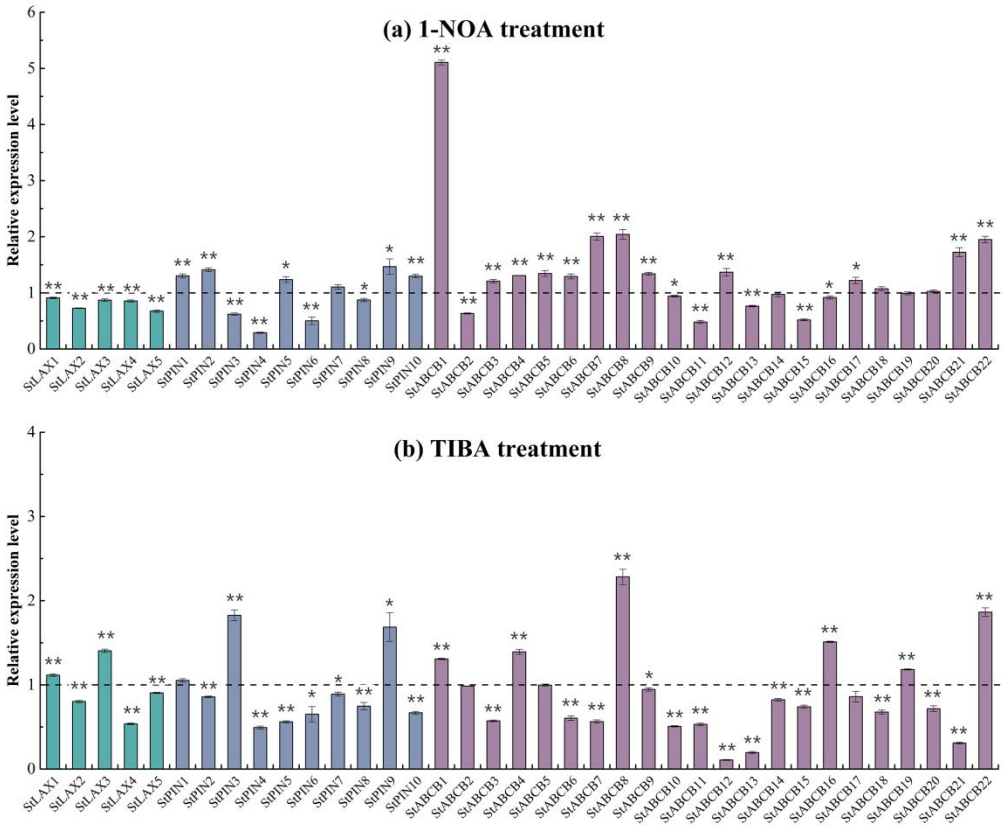


Fig 6. Expression profiles analysis of auxin transporter genes StLAX, StPIN, and StABCB under auxin transport inhibitor treatments. Potato plantlets grown at 4-week-old were treated with 30μM 1-NOA **(a)** or 50μM TIBA **(b)** for 3h. The relative expression levels were normalized to a value of 1 in the untreated seedlings. Assays were run in triplicates, and bars represent SDs. (* t-test *P*-value <0.05, **t-test *P*-value <0.01).

Expression of StLAX, StPIN, and StABCB genes in response to ABA and abiotic stresses

ABA is known as the plant 'stress hormone' and is of predominant significance due to the important role it plays in mediating both biotic and abiotic stress responses in plants [69]. Current evidence has confirmed the specific roles for auxin as a regulator of environmental adaptation in plants [70]. Additionally, the dynamic subcellular trafficking and polarity of PIN proteins are both regulated by a number of environmental responses, thus leading to a complex mechanism that integrates PINs and auxin distribution [8]. To address the possible involvement of *S. tuberosum* auxin transporters in response to abiotic stress at the transcriptional level, qRT-PCR was performed to investigate the expression fluctuations of 37 auxin transporter genes in response to ABA, salinity (NaCl), and drought (PEG) treatment compared to that in untreated plantlets grown in nutrient solution as controls. The data indicated that the majority of genes were responsive to the three stress treatments (**Figure 7; Table S8**). The majority of StLAX, StPIN, and StABCB genes, with the exception of StPIN2, StPIN5, StPIN8 and StABCB1, StABCB6, StABCB8, StABCB12, StABCB17, and StABCB18, were inhibited by ABA (**Figure 7a**). In response to salt treatment, 3 of 5 StLAXs (StLAX1, StLAX3, and StLAX5), 4 of 10StPINs (StPIN1, StPIN2, StPIN6, and StPIN9), and 5 of 22 StABCB genes (StABCB4, StABCB8, StABCB9, StABCB16, and StABCB21) were significantly down-regulated compared to levels in the control. In contrast, the rest of the three family genes were all induced by the application of NaCl, and even one or more were strongly up-regulated, including StLAX4, StPIN10, StABCB1, StABCB10, StABCB14, StABCB18, StABCB20, and StABCB22 (**Figure 7b**). In a similar

manner, half of the *S. tuberosum* auxin transporter genes were repressed by PEG treatment, and most of them were notably down-regulated (**Figure 7c**). Interestingly, the transcription of several StABCB family genes (StABCB1, StABCB11, StABCB14, StABCB15, StABCB18 and StABCB20) were dramatically up-regulated in response to PEG treatment. Overall, almost all of the *S. tuberosum* auxin transporter genes were transcriptionally mediated by stress hormone ABA, salt, and/or drought treatments, implying that the function of these genes may be associated with abiotic stress responses and adaption. The differential expression profiles of StLAX, StPIN, and StABCB genes indicated that the abiotic stress adaptive mechanism of plants was highly complex. Despite this complexity, a clear relationship between auxin transporter genes and abiotic stresses has been reported in the model plant *Arabidopsis*. The auxin influx mutant *aux1* was sensitive to high salt, where stress-induced lateral root elongation was completely blocked [71]. Additionally, salt stress altered the expression and localisation of the PIN2 protein, resulting in reduced gravity response of root growth in *Arabidopsis* [72]. To date, the effects of hormone and abiotic factors on ABCB gene expression have been reported partially and included the finding that ABA reduced the expression of AtABCB4 and its close homolog AtABCB21 [60, 73]. The expression of PGP19 was suppressed by the activation of phytochromes and cryptochromes in *Arabidopsis* [48]. In the present study, we highlighted the responsiveness of StLAX, StPIN, and StABCB auxin transporter genes to hormones and abiotic stresses. Based on this preliminary research, further functional characterisation of each transporter against abiotic stress will be performed using overexpression/knock-out transgenic studies of auxin transporters to help to elucidate the regulatory mechanisms of auxin-abiotic stress signalling.

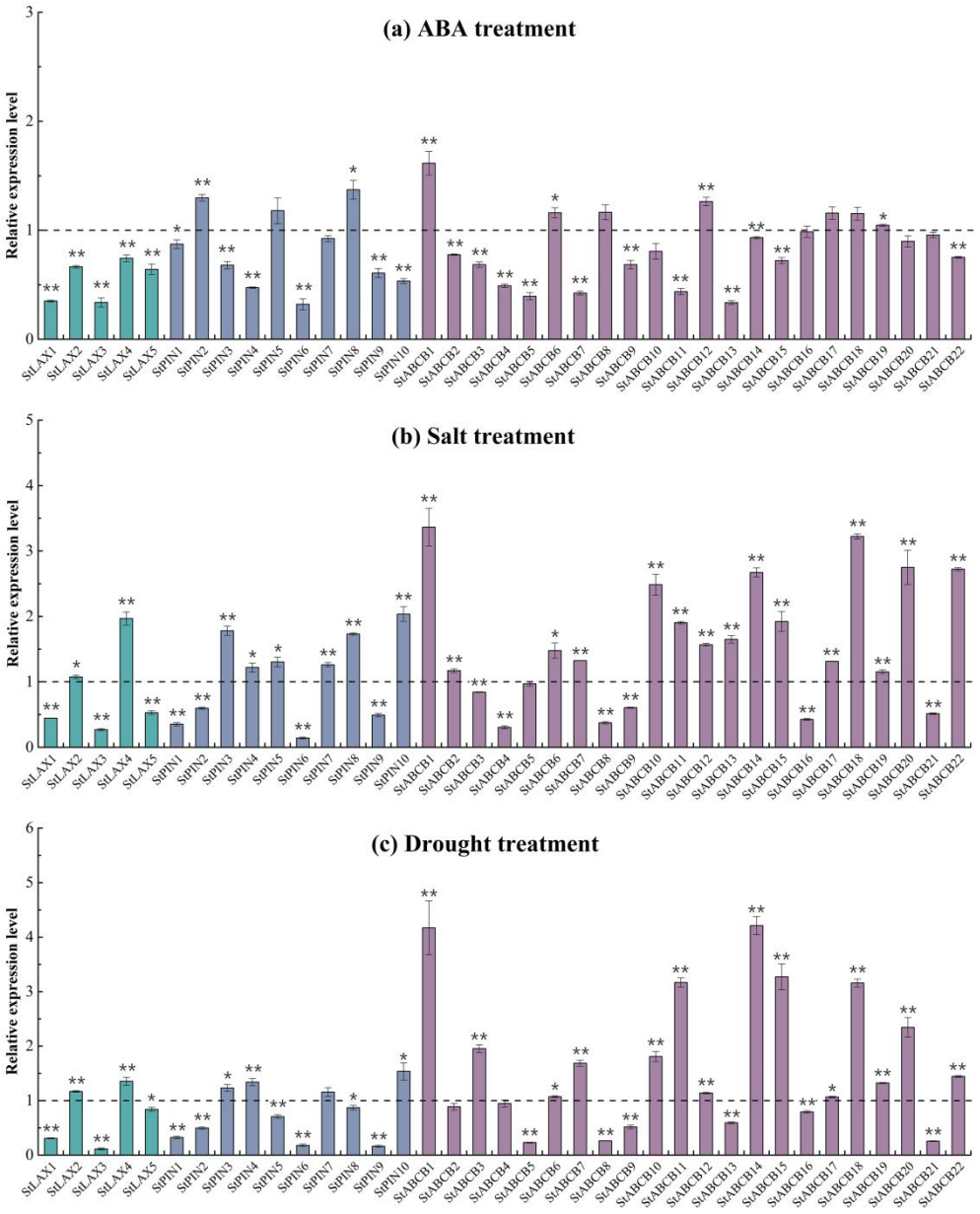


Fig 7. Expression levels of StLAX, StPIN, and StABCB family genes in response to ABA and abiotic stress. Total RNA was extracted from 4-week-old potato plantlets treated with 100μM ABA **(a)** for 3h, 200mM NaCl **(b)** or 20% (W/V) PEG (drought) **(c)** for 24h. The relative mRNA level of each gene was normalized with respect to the internal reference gene (ef1-a). The data were analyzed by three biological repeats, and SDs were shown with error bars. (* t-test *P*-value <0.05, **t-test *P*-value <0.01).

Analysis of *cis*-regulatory elements in StLAX, StPIN, and StABCB gene promoters

Cis-regulatory elements located within promoter regions play a

decisive role in the transcriptional regulation of their target genes [74]. To identify potential regulatory relationships among auxin transporter genes in potato and c*is*-regulatory elements located in their promoter regions, we retrieved the putative promoter sequences (2000bp upstream the 5'UTR region) of StLAXs, StPINs, and StABCBs from Phytozome 12.1.6 for use in scanning of designated c*is*-regulatory elements. According to the statistical results, a total of 610 auxin-responsive and stress-related c*is*-regulatory elements were detected in variable numbers (**Figure 8; Table S9**). Auxin-regulatory *cis*-elements, including AuxRE (TGTCTC), bZIP response elements (ZREs), Myb response elements (MREs), ABA responsive elements (ABREs), and other abiotic and biotic responsive elements, could account for the response of StLAXs, StPINs, and StABCBs to most external stimuli. Of these, almost all genes in the three families possessed one or more auxin-regulatory *cis*-elements in their promoter regions, and these elements may be associated with their role in auxin transport. Notably, biotic stress responsive elements (W box) occurred at a high frequency of 2 to 26 sites at each promoter, and this prompted us to speculate that all of 37 *S. tuberosum* auxin transporter genes may be involved in adaption to biotic stress. Additionally, we found that many auxin transporter genes in potato seemed to have similar *cis*-elements in their promoter regions while their expression profiles differed from one another. Presumably, there was no correlation between occupancy patterns of *cis*-elements and gene expression profiles. Even in yeast, Gao et al. confirmed the same conclusion [75]. It could be that the c*is*-regulatory elements were targeted by several transcription factors such as the ARF binding site (AuxRE:TGTCTC), the WRKYs binding site (W box:TTGAC/TGACT), the bZIPs binding site (ABRE:ACGTG), the bHLHs binding site (MYCR:CACATG), the MYBs binding site (MYBR:CTAACCA), and other homeodomain proteins [76], indicating that a complex regulatory mechanism controls a number of these transcription factors that co-mediate the expression of StLAX, StPIN, and StABCB genes.

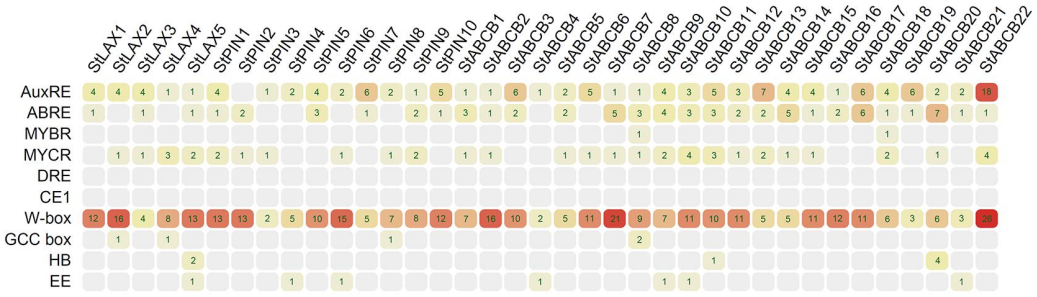


Fig 8. Analysis of auxin-responsive and stress-related *cis*-regulatory elements in the 2-kb promoter regions of StLAX, StPIN, and StABCB genes.

Conclusions

In summary, we have provided comprehensive information on StLAX, StPIN, and StABCB auxin transporter gene families in potato, which included basic parameters, chromosomal distribution, phylogeny, co-expression network analysis, gene structure, tissue-specific expression patterns, transcription analysis under exogenous hormone stimuli and abiotic stresses, and *cis*-regulatory element prediction. The responsiveness of StLAXs, StPINs, and StABCBs to auxin and PATIs that mediated intercellular auxin homoeostasis and redistribution. Additionally, the differential expression levels of StLAX, StPIN, and StABCB genes in response to ABA and abiotic stresses (salt and drought), suggested that these were specific adaptive mechanisms on tolerance to various environmental stimuli. Promoter *cis*-regulatory element description analyses suggested that a number of c*is*-regulatory elements within the promoters of auxin transporter genes in potato targeted by relevant transcription factors to respond to diverse stresses. We are confident that our results provide a foundation for a better understanding of auxin transport in potato, as we have demonstrated the biological significance of these family genes in hormone signalling and adaption to environmental stresses.

Methods

Identification of AUX/LAX, PIN, and ABCB auxin transporter

family genes in potato

To identify the putative AUX/LAX and ABCB genes in *S. tuberosum*, the available protein sequence data (DM_v3.4_pep_nonredundant) of potato were downloaded from the Potato Genome Sequencing Consortium (PGSC) [77]. The gene identifier of AtAUX/LAX and AtABCB were obtained from Balzan et al. [50] and sequences of AtAUX/LAX and AtABCB genes retrieved from phytozome 12.1.6 (https://phytozome.jgi.doe.gov/pz/portal.html) were served as queries to perform the Blast searches. Then the Hidden Markov Model (HMM) was used to identify target sequences obtained from the *S. tuberosum* genome. Pfam 01490 (transmembrane amino acid transporter protein) was used for the AUX/LAX family identification and Pfam 00005 (ABC transporter) and Pfam 00664 (ABC transporter transmembrane region) were used for the ABCB family. The remaining sequences were checked for further membrane transport protein domain search using InterProScan Sequence Search (http://www.ebi.ac.uk/Tools/pfa/iprscan/). All identified AUX/LAX and ABCB proteins among *S. tuberosum* genome together with 10 StPIN proteins based on Efstathios R's publication [53] were preserved for downstream analysis. Moreover, their information of molecular weights (MW) and isoelectric points (pI) were calculated by Pepstats (https://www.ebi.ac.uk/Tools/seqstats/emboss_pepstats/). The prediction of the trans-membrane helices for the StLAX, StPIN and StABCB proteins performed by TMHHM v.2.0 (http://www.cbs.dtu.dk/services/TMHMM). And protein subcellular localization was predicted by WoLF PSORT (http://www.genscript.com/psort/wolf_psort.html).

Genome distribution, phylogenetic tree construction, and promoter analysis

The chromosomal location data of StLAX, StPIN, and StABCB family genes were obtained from phytozome 12.1.6. Distinctive gene names were arranged according to the position from the top to the bottom on chromosomes 1-12. And visualization of chromosome and tandem duplications was employed using the MapChart software. Gene pairs with nucleotide sequence identities over 90% were considered as duplicated

genes, which were analyzed by DNAMAN software. The alignment contained full-length amino acid sequences of LAX, PIN, and ABCB from *Arabidopsis*, rice, tomato and potato was generated by ClustalW program with the default parameters and the resulting sequence alignments were then uploaded to construct the the unrooted neighbor-joining tree by the methods of the *p*-distance and complete deletion with a bootstrap of 1,000 replicates using MEGA 6.0 (http://www.megasoftware.net/). Additionally, the promoters (2,000bp) of StLAX, StPIN, and StABCB genes were obtained from Phytozome 12.1.6. And auxin responsive and stress-related *cis*-regulatory elements analysis were performed using New PLACE (https://www.dna.affrc.go.jp/PLACE/?action=newplace).

Weighted co-expression network construction

RNA-Seq data from different organs of *S. tuberosum* was chosen to construct a scale-free gene co-expression network. Gene expression levels were normalized to fragments per kilobase per million (FPKM) values. Network analysis was performed using the Weighted Gene Co-expression Network Analysis (WGCNA) R software package following step-by-step network construction and the module detection method. A proper power-law coefficient β was selected using the soft-thresholding method, and module stability was tested as the average correlation between the original connectivity and the connectivity from half samples that were randomly sampled 1000 times. The dynamic hierarchical tree cut algorithm was used to identify the co-expression gene modules based on the topological overlap matrix (TOM). Each module was summarized by a module eigengene (ME) through singular value decomposition. Additionally, a permutation test *p*-value was performed to estimate the correlation of differential expression of their corresponding eigengenes in the various tissues from potato plant, and a gene significance (GS) measure could also be defined by minus log of a *p*-value. Finally, a clustering diagram was plotted with the hclust function in the WGCNA package, and the heatmap for the correlation coefficient between modules was generated using the R package heatmap.3.R.

Gene structure and tissue-specific expression profiling analysis

The Gene Structure Display Server (GSDS) (http://gsds.cbi.pku.edu.cn/) was employed to identify exon-intron organizations of StLAX, StPIN, and StABCB family genes by comparing the coding sequences with their corresponding genomic sequences, which were collected from phytozome 12.1.6 (https://phytozome.jgi.doe.gov/pz/portal.html). To characterize the expression patterns of the StLAX, StPIN, and StABCB genes, we used the RNA-Seq data (DM_v4.03) [54] of various tissues of the RH89-039-16 genotype (referred to as RH) including flower, leaves, shoot apex, stolon, young tuber, mature tuber, and root tissue downloaded from PGSC. The expression level was calculated as fragments per kilobase per million (FPKM) values. The raw data of FPKM values were converted as log2 subsequently and then submitted to HemI [78] for establishing the expression heat maps of hierarchical clustering of the StLAX, StPIN, and StABCB genes.

Plant growth, treatments, and collection of tissues

The potato cultivar *Desiree* was used in this study. The plantlets were grown in Murashige and Skoog (MS) medium containing 2% sucrose and 0.8% agar and 0.05% MES (2-Morpholinoethanesulfonic Acid) at pH 5.8. All plantlets were grown in a plant growth chamber with a 16h light (10000Lx) and 8h dark (0Lx) photoperiod at 22±1℃. Then the four-week-old plantlets were transferred into containers of 2% MS nutritional liquid medium again and sustained for four weeks under the same growth conditions as before. Potato plantlets with consistent growth vigour were subjected to the phytohormone and abiotic stress treatments. For hormone treatments, four-week-old plantlets were soaked in 2% MS nutritional liquid medium with 10 uM IAA, 50 uM TIBA, 30 uM 1-NOA, and 100 uM ABA then incubated for 3 hours. For stress experiments, the roots of potato plantlets were immersed in nutritional liquid medium containing 200 mM NaCl or 20% (W/W) Polyethylene glycol (PEG6000) for 24 hours. Untreated plantlets were used as controls. The whole treated and control potato plantlets were collected for RNA extraction. For each treatment condition, three biological replicates were established to reduce

the error rate and a collection of samples from four potato plantlets were used as one biological replicate.

RNA isolation and qRT-PCR analysis

Total RNA from whole in vitro-grown plantlets was extracted using the high purity total RNA rapid extraction kit (TIANGEN, Beijing, China) based on manufacturer's instructions. Gel electrophoresis was used to assess RNA quality and quantity. First-strand cDNA was synthesized from 2ug of total RNA using the Fast Super RT Kit cDNA with gDNase (TIANGEN, Beijing, China). The primers sequences of individual gene families for qRT-PCR analysis were designed with Primer Primer 5 software and confirmed their specificity of unique and appropriate cDNA segments by uploading to the BLAST program (**Table S10**). qRT-PCR was performed on the Q7 Real Time PCR System using 2xRealStar Green Fast Mixture (GenStar, Beijing, China) with elongation factor 1- α (ef1- α) as the internal reference gene for normalization of gene expression [79]. The reaction was carried out in a total volume of 10 μ l containing 0.4 μ L cDNA as template, 5 μ L RealStar Green Fast Mixture (2x), 0.4 μ L of each forward and reverse primer (10 μ M), and RNase-free water up to 10 μ L. In qRT-PCR experiments the following thermal cycling conditions were applied, initial activation 95℃ for 2min, then 40 cycles of 95℃ for 15s, 55℃ for 15s and 72℃ for 19s. The melting curve generated from 65℃ to 95℃ with increments of 0.5℃ every 5s was performed to check specific amplification. The relative RNA levels of each gene were calculated from cycle threshold (C_T) values according to the $2^{-\Delta\Delta CT}$ method [80]. The data were analysed using SPSS software (SPSS version 19.0, SPSS, Chicago, IL, USA), using descriptive statistical tests; one-way analysis of variance was used to evaluate the differences between treatments and control. Statistical significance was established at 0.05 and 0.01, respectively.

Additional files

Additional file 1: Fig S1. Transmembrane topology prediction for the StLAX (A), StPIN (B), and StABCB (C) proteins. The trans-membrane helices were predicted using the TMHHM v.2.0

(http://www.cbs.dtu.dk/services/TMHMM) and the predicted trans-membrane helices was shown as red peaks.

Additional file 2: Table S1. The primer sequences used in qPCR analyses.

Additional file 3: Table S2. Percent ORF nucleotide (bottom-left) and amino acid (up-right, bold) identities of StLAXs.

Additional file 4: Table S3. Percent ORF nucleotide (bottom-left) and amino acid (up-right, bold) identities of StPINs.

Additional file 5: Table S4. Percent ORF nucleotide (bottom-left) and amino acid (up-right, bold) identities of StABCBs.

Additional file 6: Table S5. Protein sequences used in the phylogenetic relationship analysis.

Additional file 7: Table S6. Gene modules and significance *p*-value of auxin transporters in potato.

Additional file 8: Table S7. FPKM values of auxin transporter genes in various tissues.

Additional file 9: Table S8. Raw data of auxin transporter genes under IAA and PATIs treatments.

Additional file 10: Table S9. Raw data of auxin transporter genes under ABA and abiotic stresses.

Additional file 11:Table S10. Motif sites of *cis*-elements within the promoters of auxin transporter genes.

Authors' contributions

Q.C., H.M. and C.Y. conceived and designed the research plans; D.W., M. Y. and N.K. participated in most of the experiments and data collection; C.Z., M.Y. and N.K. provided technical assistance to C.Y.; C.Y. wrote the manuscript with contributions from all the authors; H.M. and C.Y. revised the manuscript. All authors read, reviewed and approved the final manuscript.

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Competing interests

The authors declare no competing interests.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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Conflict of Interest

None of the authors have any actual or potential conflicts of interest.

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Endnotes

[1] https://phytozome.jgi.doe.gov/pz/portal.html

[2] https://www.ebi.ac.uk/Tools/seqstats/emboss_pepstats/

[3] http://www.cbs.dtu.dk/services/TMHMM/

[4] http://www.genscript.com/psort/wolf_psort.html

[5] http://www.megasoftware.net/

[6] https://www.dna.affrc.go.jp/PLACE/?action=newplace

[7] http://gsds.cbi.pku.edu.cn/

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Figures

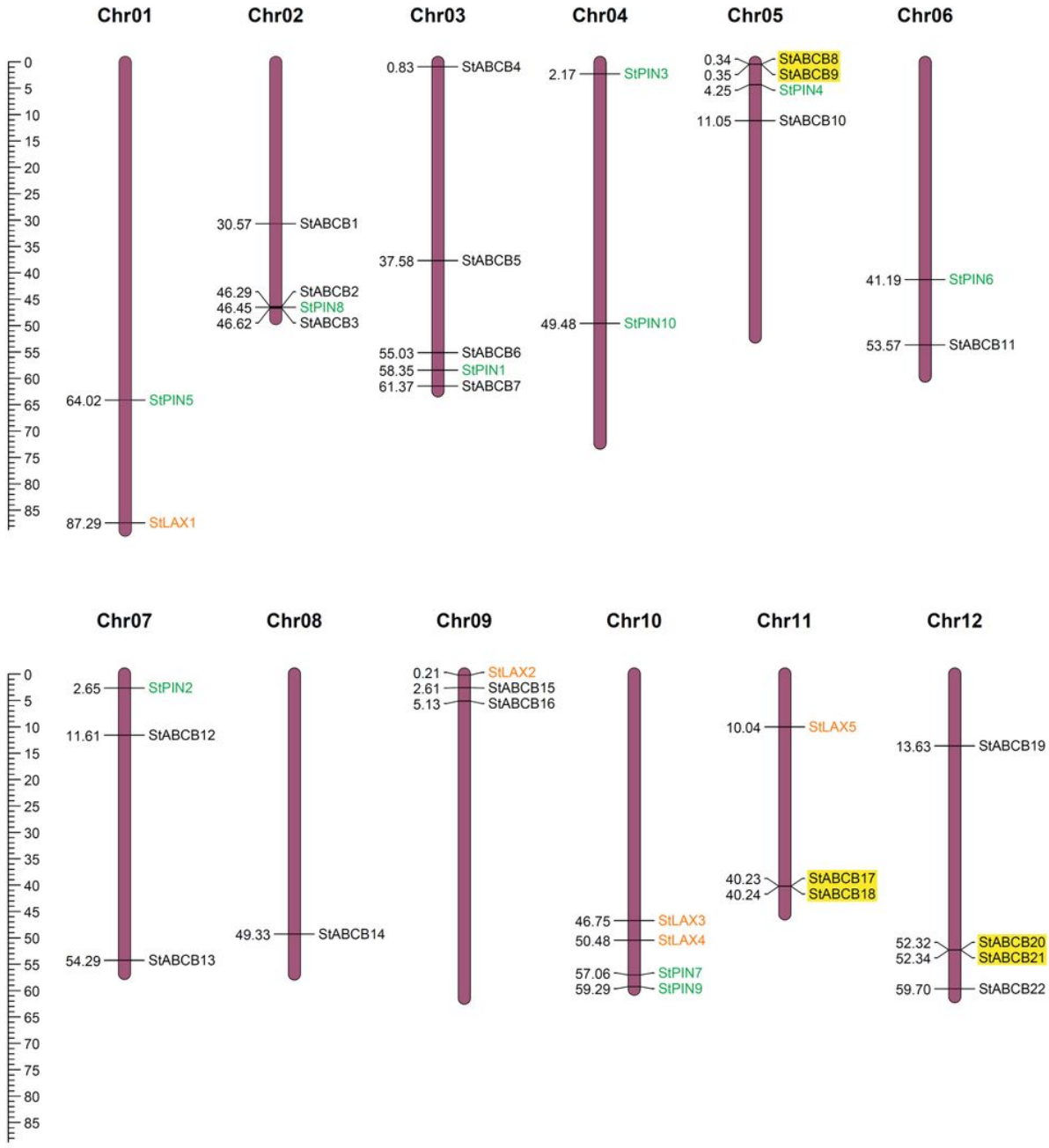

Figure 1

Chromosomal distribution of StLAX, StPIN, and 485 StABCB family genes. Potato chromosomes were arranged in blocks. Five StLAX genes, 10 StPIN genes and 22 StABCB genes were mapped by locus and the gene clusters were represented by yellow rectangles.

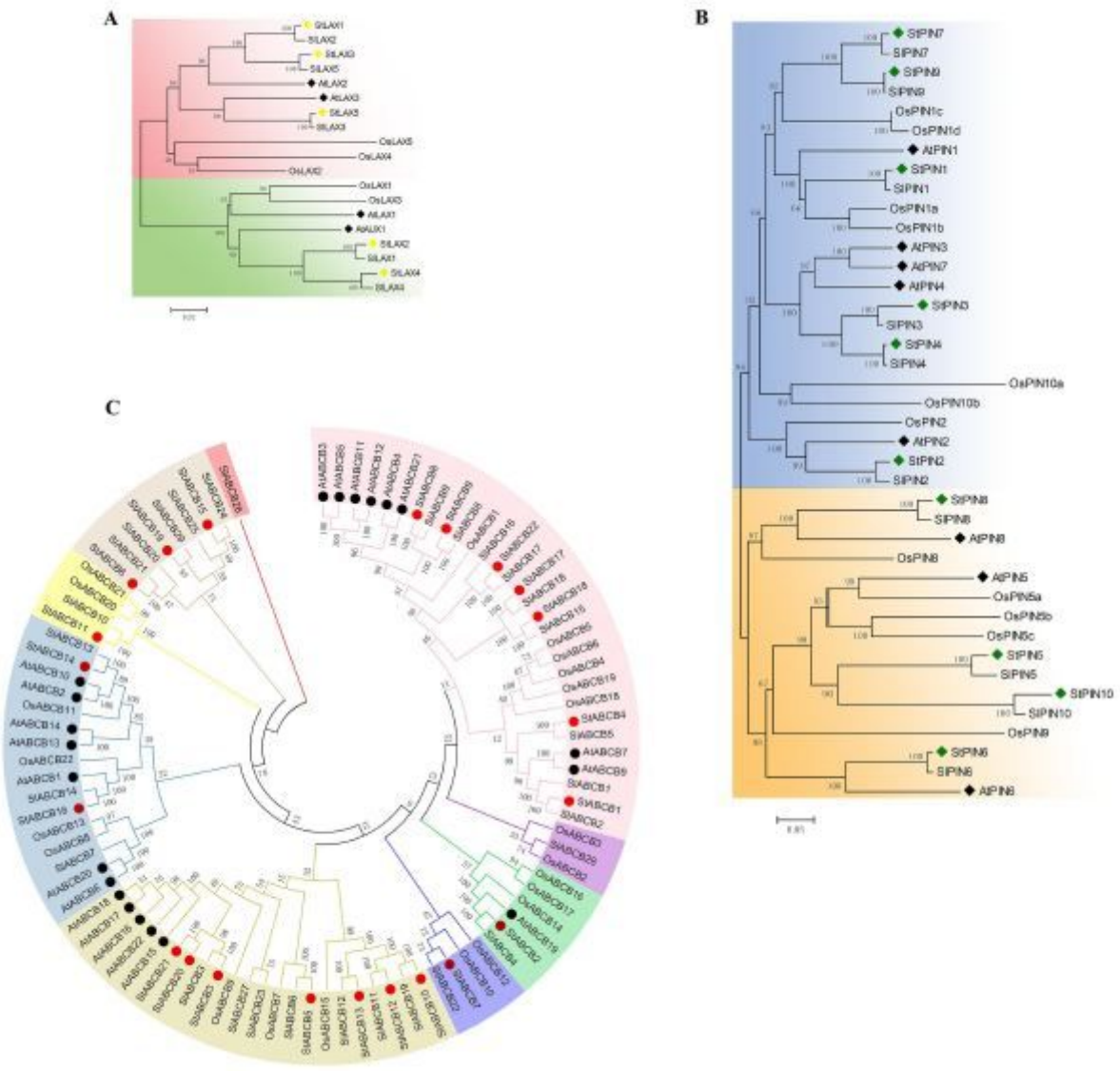

Figure 2

Phylogenetic tree of LAXs (A), PINs (B), and ABCBs 567 (C) auxin transporter protein families in Arabidopsis, rice, tomato and potato. Bootstrap values were presented for all branches. (A) LAX protein family: inventory of AtLAX families was based on TAIR databases. (B) PIN protein family: sequence data on AtPIN and StPIN families was based on TAIR annotation and Efstathios R's publication[53]. (C) ABCB protein family: inventory of AtABCB families was based on the ABC superfamily reviewed by Verrier et al[12]. Different colors indicated different subfamilies.

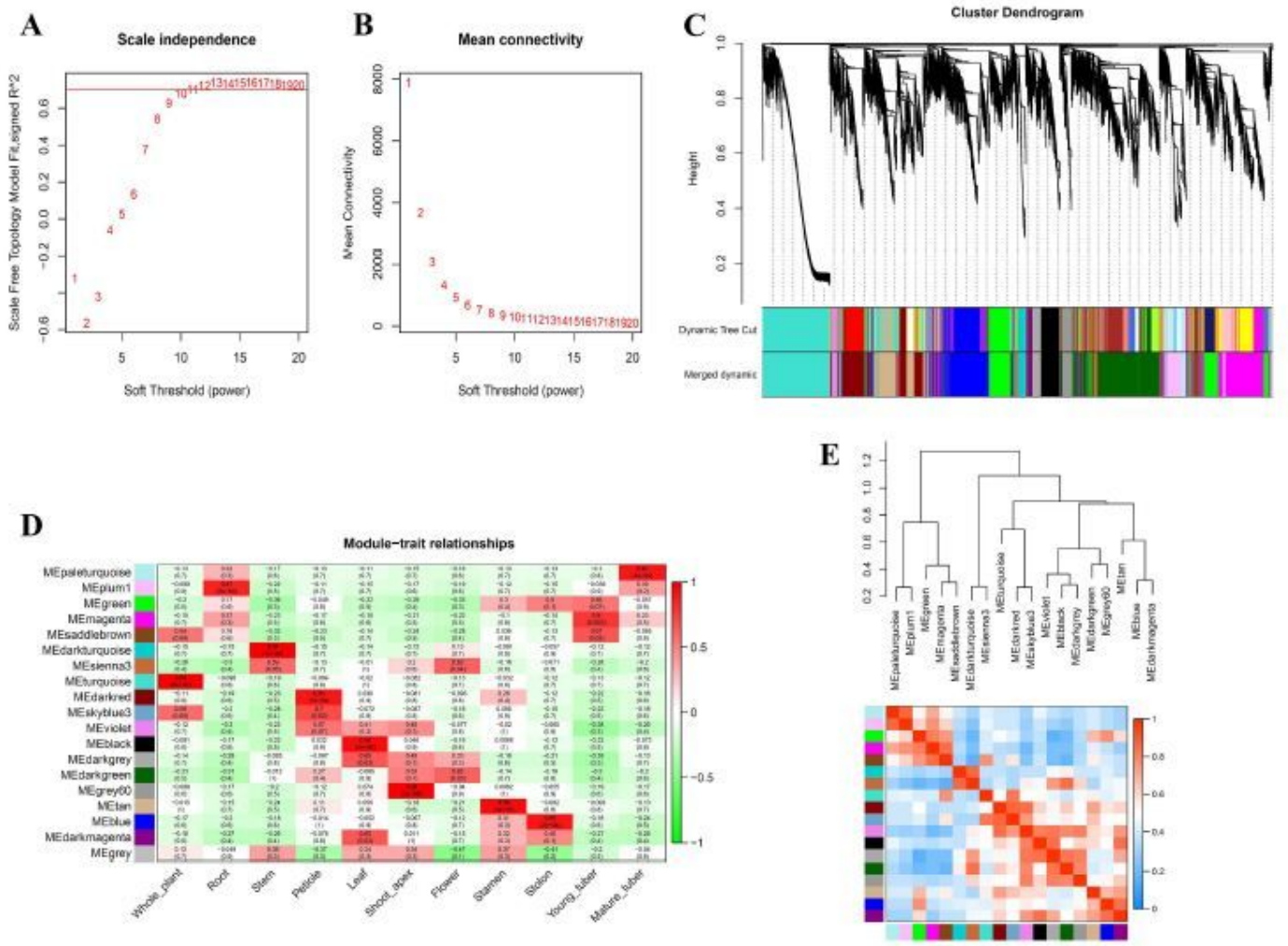

Figure 3

Graphical visualization of the S. tuberosum co-expression 613 network. (A) Plot showing the scale free topology R2 value in function of increasing soft thresholding power. (B) Plot showing the relation between mean connectivity and soft threshold. (C) Dendrograms produced by average linkage of hierarchical clustering of S. tuberosum genes, which based on a topological overlap matrix (TOM). The modules were assigned colors as indicated in the horizontal bar beneath the dendrogram. (D) Characterizing consensus modules by differential expression of their corresponding eigengenes in the various tissues from potato plant. Red meant over-expression, green meant under-expression; numbers in each cell gave the corresponding t-test p-value. Each column corresponded to a tissue and each row corresponded to an eigengene. (E) Clustering dendrograms of consensus module eigengenes for identifying meta-modules (above) and the heatmap for the correlation coefficient between the modules (below). The diagonal plots showed heatmap plots of eigengene adjacencies. Each row and column corresponded to one eigengene (labeled by consensus module color). Within the heatmap, red indicated high adjacency (positive correlation) and green low adjacency (negative correlation) as shown by the color legend.

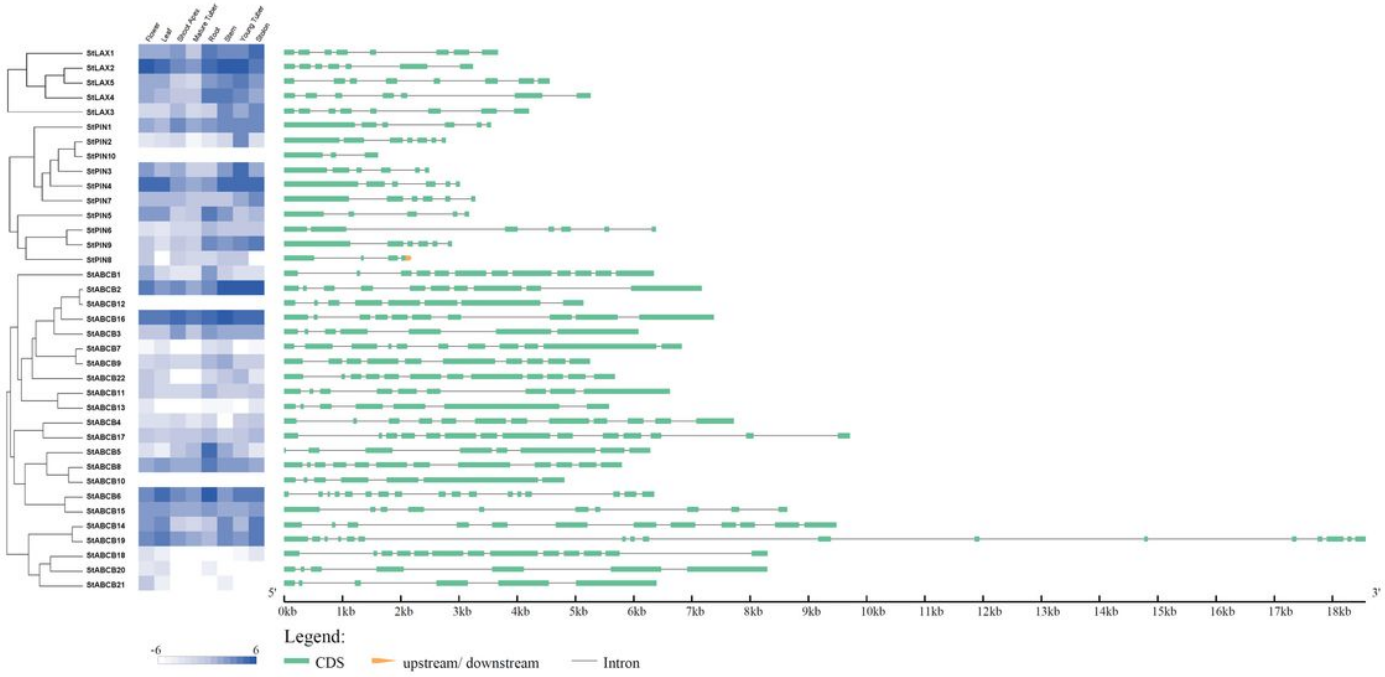

Figure 4

Tissues-specific expressions and exon-intron structures of StLAX, StPIN, and StABCB genes. The heat map was generated using the Cluster 3.0 software according to the RNA-seq data of RH. The exons were indicated by green boxes and the introns were indicated by gray lines.

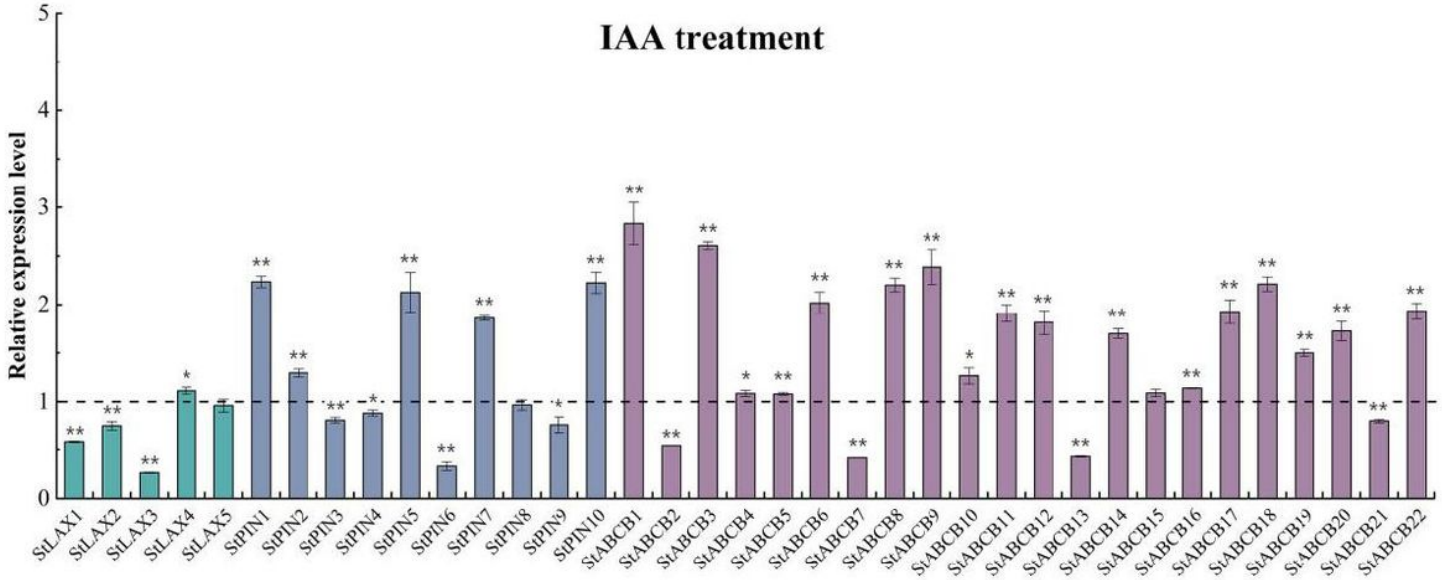

Figure 5

The relative expression values of auxin transporter StLAX, StPIN, and StABCB genes following IAA treatment. Total RNA was extracted from 4-week-old potato plantlets for expression analysis. The

histogram represents the relative RNA level of genes after IAA treatment compared with the mock expression level, which was normalized to a value of 1 . The elongation factor 1-a (ef1-a) was employed as the internal standard to normalize the relative mRNA level of individual genes. Error bars represent the standard deviations (SDs) from three biological replicates. (* t-test P-value <0.05, **t-test P-value <0.01).

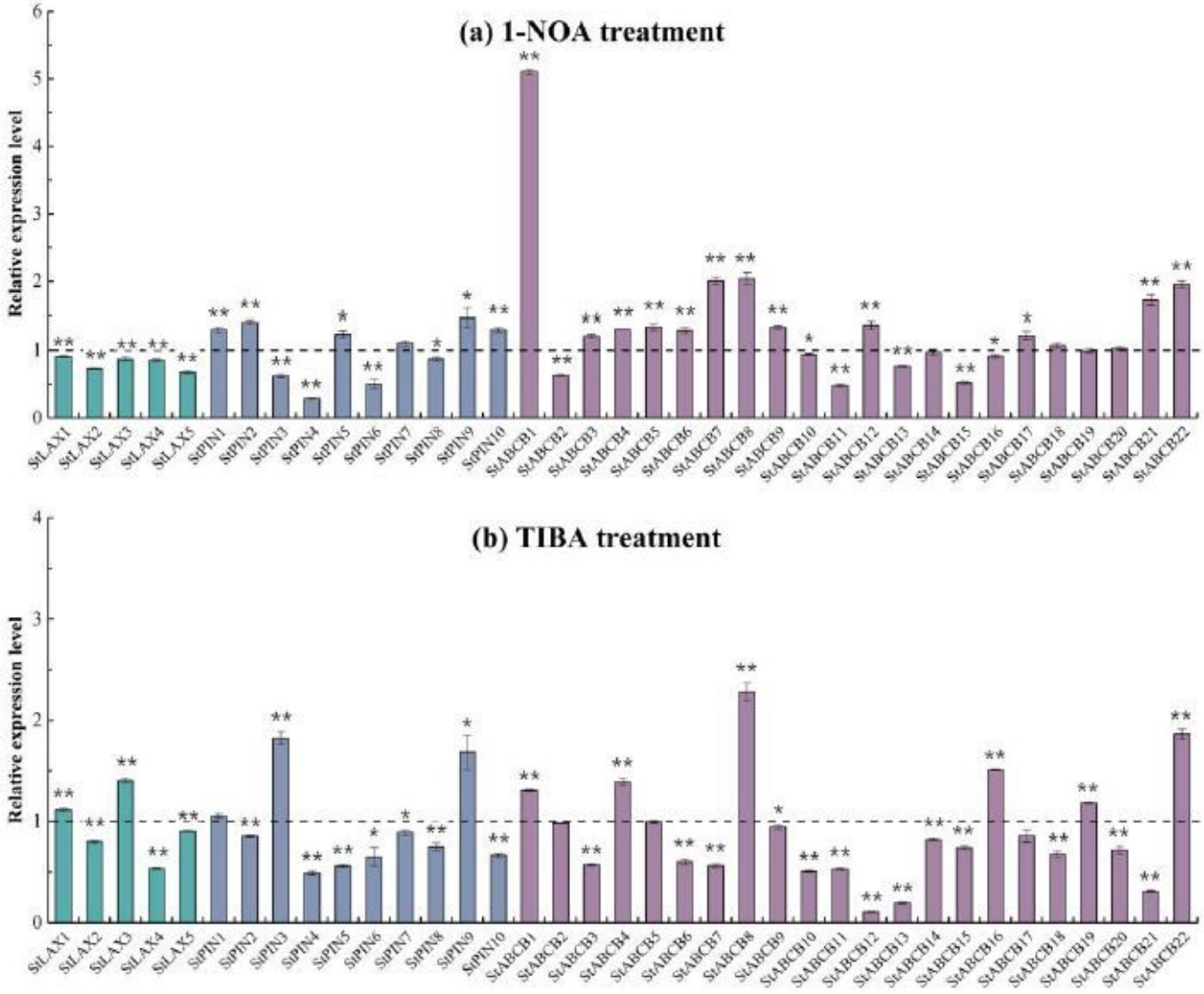

Figure 6

Expression profiles analysis of auxin transporter 766 genes StLAX, StPIN, and StABCB under auxin transport inhibitor treatments. Potato plantlets grown at 4-week-old were treated with 30μM 1-NOA (a) or 50μM TIBA (b) for 3h. The relative expression levels were normalized to a value of 1 in the untreated seedlings. Assays were run in triplicates, and bars represent SDs. (* t-test P-value <0.05, **t-test P-value <0.01).

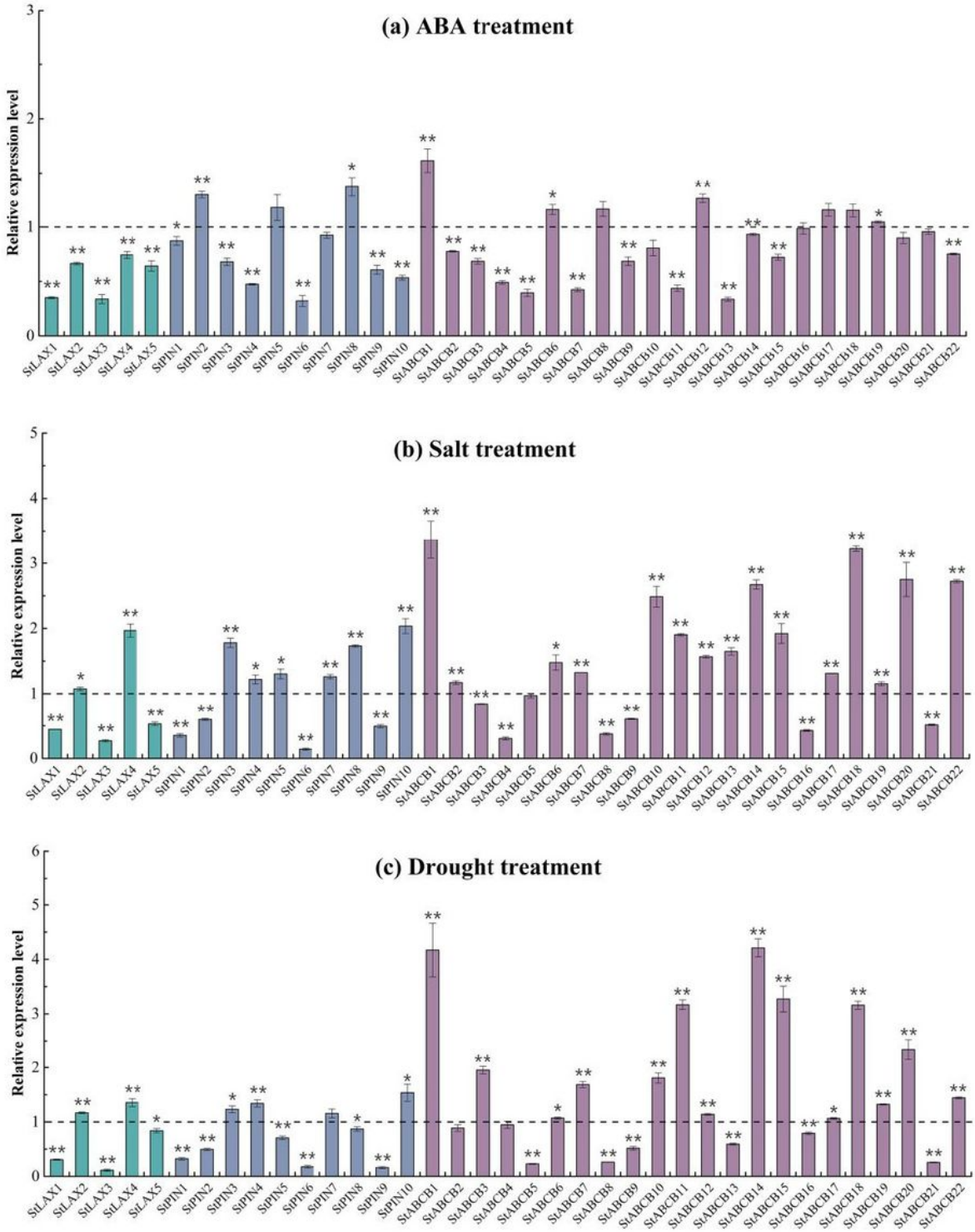

Figure 7

Expression levels of StLAX, StPIN, and StABCB family 828 genes in response to ABA and abiotic stress. Total RNA was extracted from 4-week-old potato plantlets treated with 100μM ABA (a) for 3h, 200mM NaCl (b) or 20% (W/V) PEG (drought) (c) for 24h. The relative mRNA level of each gene was normalized with respect to the internal reference gene (ef1-a). The data were analyzed by three biological repeats, and SDs were shown with error bars. (* t-test P-value <0.05, **t-test P-value <0.01).

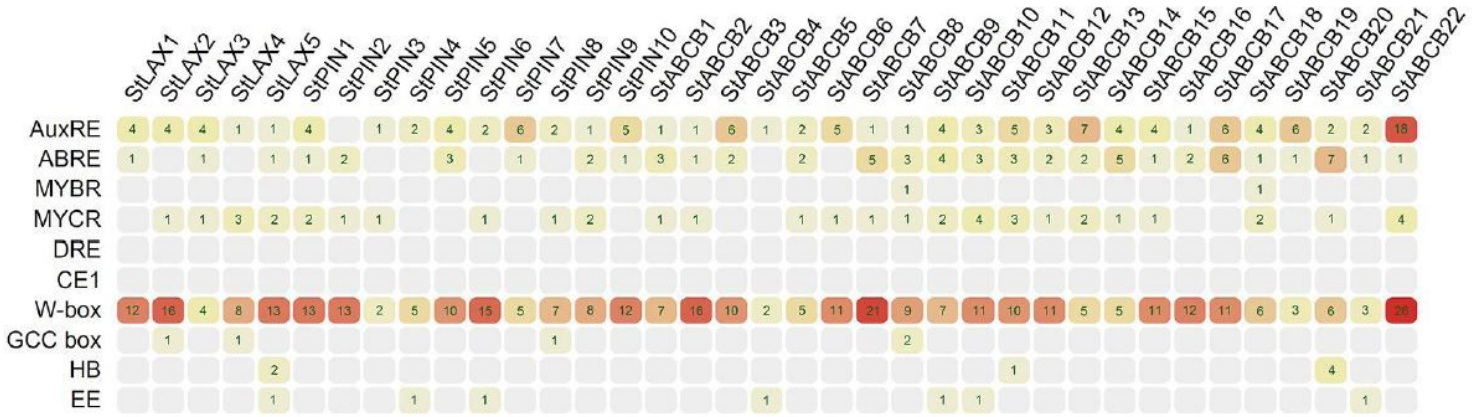

Figure 8

Analysis of auxin-responsive and stress-related cis-870 regulatory elements in the 2-kb promoter regions of StLAX, StPIN, and StABCB genes.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.xlsx
- FigureS1.tif
- TableS1.docx
- TableS10.docx
- TableS2.docx
- TableS3.xlsx
- TableS4.docx
- TableS5.xlsx
- TableS6.xlsx
- TableS7.xlsx
- TableS8.xlsx
- TableS9.xlsx