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Genome-Wide Systematic Characterisation and Expression Analysis of Expansin Gene Family Provides Insights into Their Promising Functions in Tuberous Root Development and Stress Response in Sweetpotato (*Ipomoea batatas*)

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Abstract: Expansins (EXPs) which are the essential components of plant cell wall function as relaxation factors to directly promote turgor-driven expansion of cell wall and control plant growth and development and diverse environmental stress responses. A lot of EXPs genes have been widely identified and characterized in numerous plant species, but not in sweetpotato. In the present study, a total of 59 EXPs genes that unevenly distributed onto 14 of 15 chromosomes were identified from sweetpotato genome, and segmental and tandem duplications were exhibited to act as dominant functions in IbEXPs diversity. Phylogenetic analysis exhibited that IbEXPs members were clustered into four subfamilies according to EXPs in *Arabidopsis* and rice and the regularity of motif, domain and gene structures was in accordance with the subfamily classification. The collinearity analysis between *IbEXPs* and related homologous sequences in 9 plants provided the phylogenetic insights of EXPs gene family. Cis-element analysis futhrer revealed the potential roles of *IbEXPs* in sweetpotato development and stress responses. Evidence from RNA-seq and qRT-PCR analysis of eight selected *IbEXPs* genes displayed their various tissue specificity in different tissues, and their transcripts were varyingly induced or suppressed under multiple hormone treatments (ABA, SA, JA and ACC) and abiotic stresses (low and high temperature). These results supply worthy foundations to further comprehensively investigate the exact functions of *IbEXPs* genes, and multiple members may function as promising regulators to control plant development and enhance the stress resistance of transgenic plants.

Keywords: expansin gene family; systematic characterization; expression analysis; tuberous root development; abiotic stress; hormones; sweetpotato

1. Introduction

Among the variousplant cell components, the cell wall which is a kind of distinct, crucial and dynamic structure has the good extensibility. It determines and maintains the cell size and shape and serves as the protective barrier [1–3]. The plant cell wall is highly complex structures constituted by various polysaccharides that vary in abundance, function, and structure [4]. The cell wall plays crucial roles in supplies of stiffness and mechanical support to plant body, resistance to abiotic and biotic stresses, conduction of nutrients and water, determination of plant architecture and morphogenesis [5]. Nowdays, the study on cell wall extension mechanism has become the research

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priority due to the significance of cell wall enlargement during plant morphogenesis [6]. The increase of cell volume and quantity which hinges on the cell wall enlargement and loosening is crucial for plant growth [7]. Cell wall loosening is the significant precondition of cell wall remodeling, in which the physical structure is altered or new components are added into the cell wall, inducing alteration of shape and anisotropic growth in the cell [7]. The modified proteins which attach to the cell wall serve as the vital roles in cell wall enlargement and loosening, the most widely recognized of which are the expansins (EXPs) [8].

EXPs are the crucial proteins related to cell wall that participate in cell wall enlargement and loosening and are commonly found in plants [9]. As the primary factor of enlargement and loosening, EXPs can control the cell relaxation without any chemical energy through the non-enzymatic activity [7]. Expansin can act directly on plant cell wall to loosen the cell wall via binding to the cellulose in cell wall to disrupt hydrogen bonds which exist in wall matrix polysaccharides and cellulose microfibrils in the pH-dependent manner, and also take part in the decomposition, remodeling, extension and assembly of cell wall [7,9–11]. Plant expansins usually are composed of a signal peptide (SP) at the N-terminal (about 20-30 amino acid residues) and two domains. Domain I is a six-stranded double-psi beta-barrel (DPBB) and locates at the N-terminal. It shares the homologous with the catalytic domain of GH45 proteins (glycoside hydrolase family 45) and harbors a conserved His-Phe-Asp (HFD), but do not has the β-1, 4-glucanase activity [12]. This region is rich in cysteine (Cys) residues with one characteristic catalytic domain which may have something to do with the disulfide bond formation [13]. The Domain II which harbors a β-sandwich fold and shares about 50% similarity with the group-II pollen allergen protein (pollen_allerg_1, G2A family) is considered as the polysaccharide binding domain due to it cantains the conserved aromatic amino acids and polar tryptophan residues on its surface. It cantains 90-120 amino acid residues and was classified as the family-63 carbohydrate binding module (CBM63) [7,14].

According to the standardized nomenclature and phylogenetic analysis, plant EXPs proteins are divided into 4 subfamilies: EXPA (α-expansin), EXPB (β-expansin), EXLA (expansin-like A) and EXLB (expansin-like B) [15,16]. Nowadays, numerous EXPs members of this four subfamilies have been identified in numerous plants. Among them, the roles of EXPA and EXPB have been widely studied, which show the wall-loosening activities to participate in cell expansion and plant developmental processes [15,16]. While members of EXLA and EXLB mainly play functions in stress response, hypocotyl length and root architecture [17–19]. Based on the previous investigations, EXPs are regarded as the main determinant of cell shape in many cell developmental processes, especially in the regulation of cell-wall extensibility [20–22], including elongation and expansion [23]. Since the first identification in cucumber hypocotyl [24], EXPs proteins have been commonly found in numerous plant species.

It has been widely shown that EXPs play crucial functions in multiple biological processes by cell-wall modification and elongation, such as biotic and abiotic stress, the root and fiber development, root nodule formation, fruit development and ripening, and other developmental processes [7,10,20,22]. For instance, overexpression of *Osmanthus fragrans OfEXLA1* gene increased the resistance to salt and drought stress in *Arabidopsis* [25]. Ectopic ovexpression of wild *Arachis AdEXLB8* in tobacco increased tolerance to biotic (*Meloidogyne incognita* and *Sclerotinia sclerotiorum*) and abiotic (drought) stresses [26]. The wheat *TaEXPA2* can significantly elevate the resistance of transgenic plants to Cd toxicity and multiple abiotic stresses (drought, oxidative and salt) [27–30]. Ectopic expression of poplar *PttEXPA8* in tobacco enhanced the heat resistance in transgenic plants [31]. Three expansin genes, Tomato *SlExp1*, apple *MdEXLB1*, mango *MiExpA1* have been identified as the crucial determinants during fruit softening and ripening [32–35]. Two β-Expansin Gene *GmINS1* and *GmEXPB2* play significant roles in nodule formation and development [8,36]. The rice *OsEXPB2* and *OsEXPA8*, Soybean *GmEXLB1* and *GmEXPB2* function as the important regulators in root system architecture [18,36–39]. *Stylosanthes SgEXPB1*, rice *OsEXPA10*, and *Arabidopsis AtEXPA7* are required for root development [6,40,41]. Upregulation of *GhEXPA8* or *GbEXPATR* can increase the fibre length in cotton, while reduced EXPA results in shorter fibre [42]. In addition, plant expansin

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genes also play vital roles in height and leaf growth [43,44], pollen tube and stem elongation [45–47], seed development, germination and yield [48–50], flower development [51], and so on.

As the seventh most important food crop, sweetpotato (*Ipomoea batatas*) is the only crop that generates starch storage roots among plant species in the Convolvulaceae [52,53]. It has been one of the most widely cultivated food crops worldside due to its numerous advantages, such as low input requirements, strong stress resistance, wide adaptability, high yield and starch content, and make it a significant food crop in the world [54]. At present, sweetpotato has been broadly used in alcohol and starch production, animal feed, starch processing, and human food. And it also ensures the food security in a lot of developing countries on account of its adaptation in various environmental conditions [53]. In previous study, 37 EXP genes were identified from *Ipomoea trifida*, which is the most possible diploid wild relative species of sweetpotato [55]. However, the *Ipomoea trifida* genome does not adequately represent the whole sweetpotato genome information. Recently, the completion of hexaploid sweetpotato genome sequencing provides sufficient and valuable information for the identification and characterization of gene families [56], whereas the genome-wide identification of sweetpotato EXP gene family is still lagging and so it is necessary for us to comprehensively identify and characterize the EXP gene in sweetpotato.

In the current stage of cropmolecular breeding, one of the main focuses is to improve environmental stress resistance and promote plant growth. Related investigations have been performed systematically in a variety of plant species due to the compatiblity between the crucial functions of EXPs genes and the demand of breeding. Nowadays, a large number of EXPs genes have been broadly identified from diverse plants, for instance, a total number of EXPs genes have been identified in monocots, such as 92, 58, 241, 88, 46, 38 genes in sugarcane [4], rice [15], common wheat [57], maize [58], barley [59], *Brachypodium distachyon* [60], respectively, and in dicotyledons, such as 36, 75, 46, 93, 52 genes were found in *Arabidopsis* [15], soybean [61], gingkgo [62], cotton [63], tobacco [64], respectively. The genome sequencing of hexaploid sweetpotato (Taizhong6) has been completed [56], however, no systematical identification and characterization on EXPs genes in sweetpotato (*Ipomoea batatas* L.) is available. The identification of molecular feature of significant EXPs gene family will contribute to further understand the regulatory mechanism of plant development and adaptation to environmental stresses. In this study, 59 EXPs genes which were divided into four subfamilies (36 *IbEXPAs*, 10 *IbEXPBs*, 2 *IbEXLAs*, 11 *IbEXLBs*,) were indentified from sweetpotato genome. To standardize the nomenclature of EXPs proteins in sweetpotato and evaluate their possible functions and relationships in development and stress responses, the comprehensively systematical characterization of 59 identified sweetpotato EXPs genes was conducted. The phylogenetic relationship, chromosomal location, conserved motif and domain, gene structure, molecular characterization, cis-element, gene duplication and the expression pattern analysis of *IbEXPs* in different tissues and various treatments of hormones and abiotic stresses were investigated in this study. All data obtained in this study will lay the worthy foundation for the further screening and functional investigation of valuable EXPs genes that play crucial roles in tuberous root development and stress tolerance in sweetpotato.

2. Results

2.1. IbEXP gene identification and characterization in sweetpotato

In present study, a total number of 59 IbEXP genes were identified from sweetpotato genome and were named following the AtEXPs and OsEXPs classification in *Arabidopsis* and and rice their position on chromosome. These 59 IbEXP proteins were divided into four subfamilies, namely IbEXPA, IbEXPB, IbEXLA and IbEXLB, with 36, 10, 2 and 11 members, respectively. After that, the protein size (aa), theoretical isoelectric point (pI), molecular weight (Mw) and phosphorylation site of 59 IbEXP proteins were explored. The detailed data were shown in Table 1. The protein length and Mw of IbEXPs varied widely, with the length of proteins varied from 183 aa (IbEXPA31) to 670 aa (IbEXPA19) and the Mw ranging from 20.1739 KD (IbEXPB31) to 74.052 KD (IbEXPA19). The PI ranged from 4.63 (IbEXLB11) to 9.82 (IbEXPA34). The subcellular location prediction exhibited that

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most of IbEXPs were located on cell wall, and very few IbEXPs were located in nucleus (IbEXPA19), and simultaneously located in chloroplast and on cell wall (IbEXPA22 and IbEXPA33). The results of phosphorylation site prediction of IbEXPs displayed that significant changes from 24 (IbEXPA17 and IbEXPA31) to 109 (IbEXPA12), and the vast majority of IbEXPs harbored more Ser sites than that of Tyr and Thr sites. Moreover, over 83.05% of IbEXPs have at least 30 phosphorylation sites.

Table 1. Characteristics of IbEXP proteins in *Ipomoea batatas.*

Gene name	Gene ID	Amino acids	MW (Da)	PI	Subcellular location	phosphorylation cite			
						Ser site (S)	Tyr cite (Y)	Thr cite (T)	Total
IbEXPA1	g1306.t1	238	25431.71	9.53	Cell wall.	20	3	8	31
IbEXPA2	g3925.t1	251	26960.41	8.07	Cell wall.	14	4	8	26
IbEXPA3	g3926.t1	251	26740.92	8.36	Cell wall.	21	4	6	31
IbEXPA4	g4923.t1	260	28213.08	9.27	Cell wall.	26	3	7	36
IbEXPA5	g9889.t1	310	33660.23	9.25	Cell wall.	26	5	20	51
IbEXPA6	g10004.t1	258	27595.27	9.3	Cell wall.	19	3	4	26
IbEXPA7	g15786.t1	327	35627.31	9.29	Cell wall.	45	5	12	62
IbEXPA8	g15816.t1	272	29860.26	9.36	Cell wall.	25	4	12	41
IbEXPA9	g15820.t1	264	28775.77	9.37	Cell wall.	29	4	9	42
IbEXPA10	g16869.t1	263	28645.8	8.56	Cell wall.	17	4	13	34
IbEXPA11	g17886.t1	258	27852.84	9.5	Cell wall.	13	4	10	27
IbEXPA12	g18537.t1	591	63172.9	7.19	Cell wall.	77	12	20	109
IbEXPA13	g18539.t1	341	35684.91	7.05	Cell wall.	57	6	10	73
IbEXPA14	g20283.t1	260	28086.01	9.4	Cell wall.	20	2	12	34
IbEXPA15	g24580.t1	252	27413.57	6.42	Cell wall.	12	4	11	27
IbEXPA16	g25447.t1	260	27406.89	9.16	Cell wall.	45	3	8	56
IbEXPA17	g25606.t1	209	22967.29	9.73	Cell wall.	13	3	8	24
IbEXPA18	g29706.t1	355	39001.06	9.64	Cell wall.	47	2	9	58
IbEXPA19	g29707.t1	670	74052.16	8.27	Nucleus.	72	5	23	100
IbEXPA20	g30029.t1	202	22488.71	9.44	Cell wall.	12	2	12	26
IbEXPA21	g33328.t1	273	28718.04	6.2	Cell wall.	30	5	7	42
IbEXPA22	g38554.t1	493	53544.79	8.24	Cell wall, Chloroplast	53	9	7	69
IbEXPA23	g39420.t1	309	33789.29	9.29	Cell wall.	29	4	20	53
IbEXPA24	g39467.t1	270	29209.17	9.1	Cell wall.	26	5	11	42
IbEXPA25	g42794.t1	248	26037.26	9.34	Cell wall.	16	2	7	25
IbEXPA26	g47278.t1	246	25903.09	8.64	Cell wall.	18	4	3	25
IbEXPA27	g53361.t1	253	27102.64	8.87	Cell wall.	22	3	6	31
IbEXPA28	g53365.t1	563	61262.75	9.32	Cell wall.	56	5	25	86
IbEXPA29	g58047.t1	241	26303.82	6	Cell wall.	23	3	13	39
IbEXPA30	g58048.t1	240	26373.45	9.21	Cell wall.	22	5	14	41
IbEXPA31	g58049.t1	183	20173.91	6.81	Cell wall.	10	2	12	24

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IbEXPA32	g58052.t1	388	43264.45	9.2	Cell wall.	38	2	15	55
IbEXPA33	g58053.t1	521	58831.3	6.61	Cell wall, Chloroplast	26	9	27	62
IbEXPA34	g58951.t1	257	27894.88	9.82	Cell wall.	17	2	7	26
IbEXPA35	g60585.t1	237	25641.21	9.35	Cell wall.	20	2	9	31
IbEXPA36	g61698.t1	264	28887.9	9.39	Cell wall.	28	4	10	42
IbEXPB1	g9145.t1	207	21810.79	9.49	Cell wall.	22	1	10	33
IbEXPB2	g24143.t1	243	25960.18	5.75	Cell wall.	31	4	4	39
IbEXPB3	g24144.t1	400	44133.02	7.09	Cell wall.	56	7	21	84
IbEXPB4	g27129.t1	308	33145.35	7.52	Cell wall.	47	5	20	72
IbEXPB5	g27144.t1	265	28079.59	6.19	Cell wall.	37	2	5	44
IbEXPB6	g27334.t1	342	36757.24	5.16	Cell wall.	50	5	7	62
IbEXPB7	g27336.t1	256	27320.01	8.37	Cell wall.	31	5	3	39
IbEXPB8	g48839.t1	266	27877.16	4.89	Cell wall.	35	3	8	46
IbEXPB9	g48841.t1	336	36036.73	6.69	Cell wall.	32	5	6	43
IbEXPB10	g54432.t1	262	28422.34	8.74	Cell wall.	19	1	14	34
IbEXLA1	g904.t1	266	28622.45	5.29	Cell wall.	22	4	9	35
IbEXLA2	g59839.t1	268	29409.37	6.96	Cell wall.	17	4	12	33
IbEXLB1	g14386.t1	252	28275.14	5.45	Cell wall.	12	14	10	36
IbEXLB2	g14427.t1	443	49325.01	8.93	Cell wall.	37	15	21	73
IbEXLB3	g14812.t1	351	38060.5	7.91	Cell wall.	40	10	21	71
IbEXLB4	g15079.t1	284	30949.46	8.57	Cell wall.	29	4	8	41
IbEXLB5	g15300.t1	247	26876.61	8.32	Cell wall.	20	7	6	33
IbEXLB6	g33144.t1	251	28135.78	5.41	Cell wall.	17	15	10	42
IbEXLB7	g33212.t1	278	31034.24	5.52	Cell wall.	17	13	10	40
IbEXLB8	g55672.t1	272	28559.26	6.58	Cell wall.	24	5	17	46
IbEXLB9	g55673.t1	247	27029.36	9.33	Cell wall.	30	8	18	56
IbEXLB10	g55674.t1	246	26984.94	8.46	Cell wall.	16	14	13	43
IbEXLB11	g55709.t1	308	32778.84	4.63	Cell wall.	24	7	12	43

2.2. Phylogenetic relationship of IbEXPs in sweetpotato

To explore the evolutionary relationships of IbEXPs in sweetpotato, phylogenetic analysis was carried out using the protein sequences of 59 IbEXPs, 36 Arabidopsis AtEXPs and 58 rice OsEXPs (Supplementary file S1, Table S1). Then the unrooted phylogenetic tree was constructed using the neighbor-joining bootstrap method by the MEGA software (version 11.0). Phylogenetic tree analysis exhibited that 59 IbEXPs were divided into four subfamilies according to the topology of the tree, clades support values, and reported studies about the EXPs classification in *Arabidopsis* and rice [15] (Figure 1), and the sizes of every subfamily vary greatly. The number of IbEXP members contained in these four subfamilies ranged from 2-36, with the EXPA subfamily containing the most IbEXPs (36) and the EXLA subfamily with only two members of IbEXPs, which are consistent with that in *Arabidopsis* and rice. The member differences among IbEXPs, AtEXPs and OsEXPs divided into in the same subfamily indicated that apparent interspecific divergences of EXP gene family exist among sweetpatato, *Arabidopsis* and rice.

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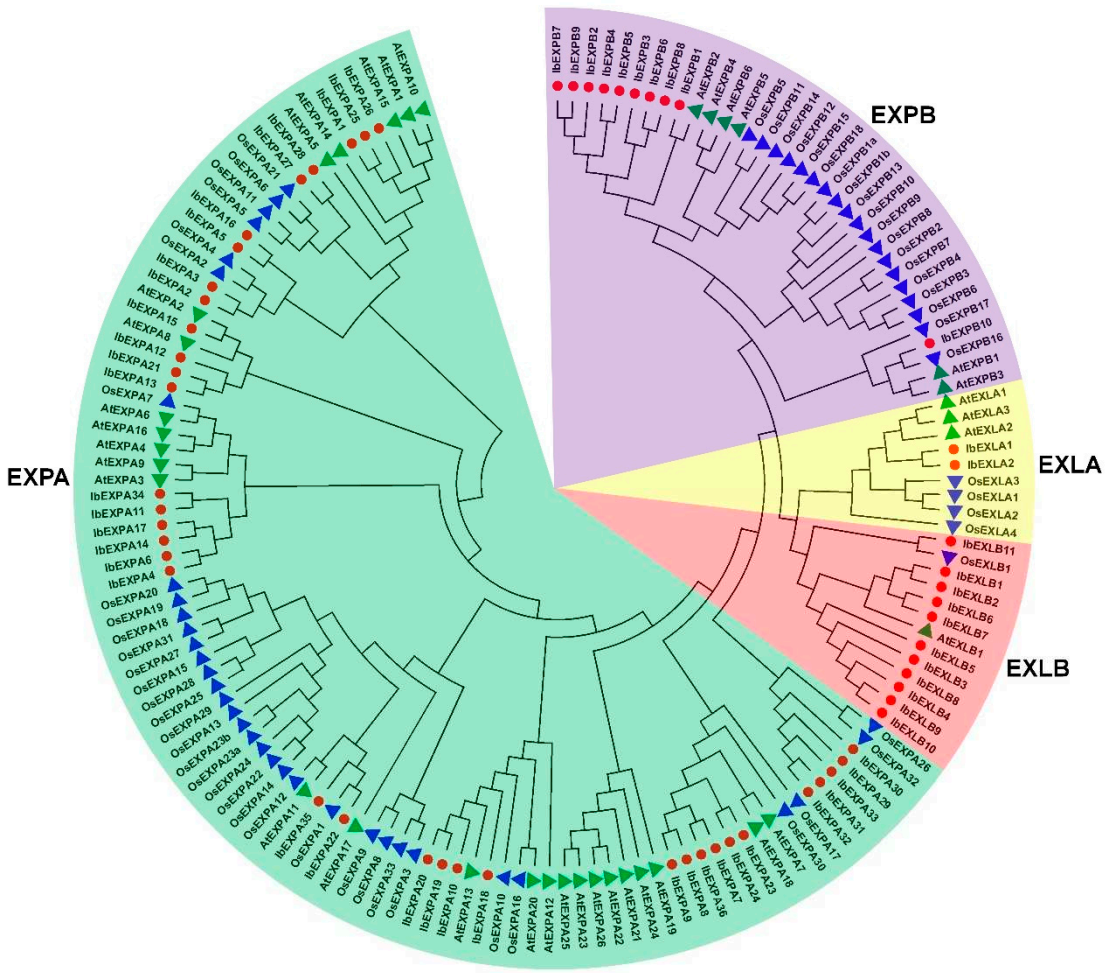

Figure 1. Phylogenetic tree of 59 IbEXPs and EXPs in *Arabidopsis* and rice. The phylogenetic relationships were constructed by the MEGA 11.0 software using the neighbor-joining bootstrap method according to the following parameters: poisson model, pairwise deletion and 1,000 replicates. Different subfamilies are named following the studies in *Arabidopsis* and rice, and different colors were used to distinguish each subfamily. Red circles, green triangles and blue triangles represent sweetpotato IbEXPs, *Arabidopsis* AtEXPs and rice OsEXPs, respectively.

2.3. Chromosome localization of sweetpatato IbEXP genes

Chromosome distribution analysis based on sweetpatato GFF3 genome annotations exhibited that 59 *IbEXPs* genes were located on 14 chromosomes of sweetpatato and no *IbEXP* genes were found on LG 9. In general, *IbEXP* genes are unevenly distributed on the 14 chromosomes, which may be the results of uneven gene replication of chromosome fragments. Among them, LG 14 contained the most *IbEXP* genes (11), and the LG 7 contained the second number of *IbEXP* genes (9), while LG 11 had only one *IbEXP* gene. Furthermore, there were eight on LG 4, five on LG 5, and two to four on other Chrs (Figure 2). These results suggested that *IbEXPs* distribution had high variable densities and was disproportionate to the length of chromosome. For instance, the largest chromosome (LG 11) contained only one *IbEXP* gene, while the smallest chromosome (LG 10) contains three *IbEXP* genes.

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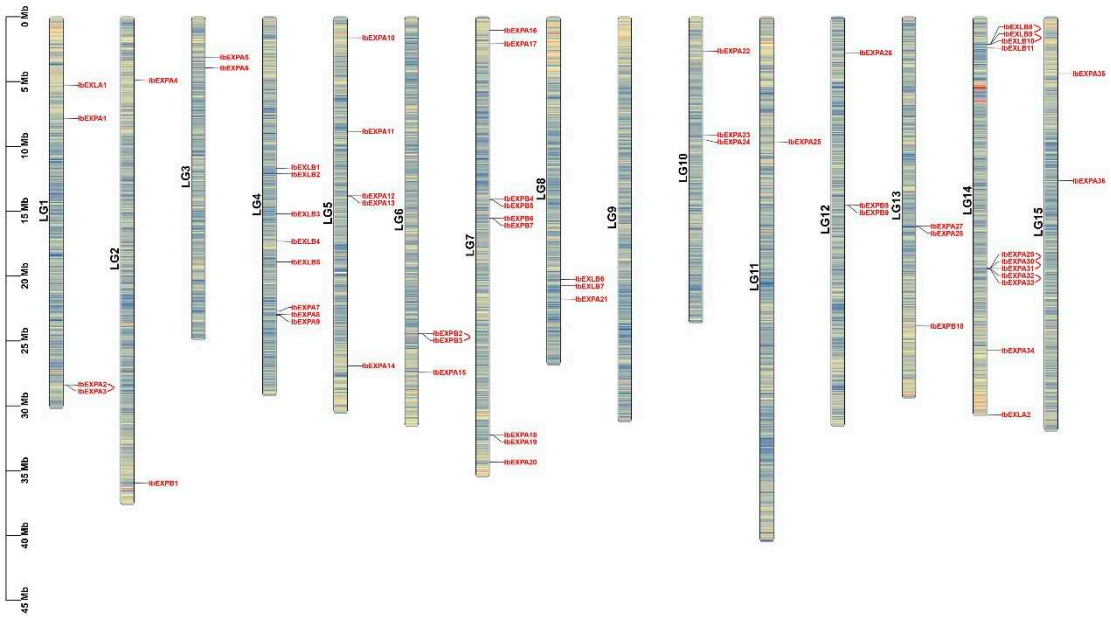

Figure 2. Localizations of 59 *IbEXPs* on sweetpotato chromosomes (LG1-LG15). The red arc behind some *IbEXPs* represent the gene duplication of part of the EXP genes in sweetpotato.

2.4. Collinearity analysis of sweetpatato IbEXP proteins

In plants, genome duplication facilitate the expansions and evolutions of gene families [65]. In order to explore the potential gene duplications amone all 59 *IbEXP* genes, the collinearity analysis of *IbEXPs* was performed using the MCScanX and BlastP programs. The results exhibited that 7 gene pairs with tandem duplication among *IbEXP* genes were indentified, including *IbEXPA2-IbEXPA3*, *IbEXPB2-IbEXPB3*, *IbEXLB8-IbEXLB9*, *IbEXLB9-IbEXLB10*, *IbEXPA29-IbEXPA30*, I*bEXPA30-IbEXPA31*, *IbEXPA32-IbEXPA33* (Figure 2, Table S2-1). And these *IbEXP* genes exhibiting tandem duplications belonged to the same subfamily. In addition, MCScanX and BlastP programs were also carried out to identify the fragment duplications and 3 gene pairs of only EXPA subfamily were found on only 5 (LG1-3, LG5, LG7) of 15 chromosomes as follows: *IbEXPA2-IbEXPA16*, *IbEXPA4-IbEXPA6*, *IbEXPA14-IbEXPA17* (Figure 3, Table S2-2). However, there were no more fragment duplications of *IbEXPA* genes were found on other then chromosomes (LG4, LG6, LG8-15) and no fragment duplications were detected in other three subfamilies. These segmental duplications occured only between genes in the EXPA subfamily may be one of the reason that the number of EXPA members was larger than that of other three subfamilies and also indicate the functions of EXPA genes in regulating plant development and response to stress were more significant than that of genes in other three subfamilies. In brief, these results suggest that gene duplication are conducive to the expansion of sweetpatato *IbEXP* gene family.

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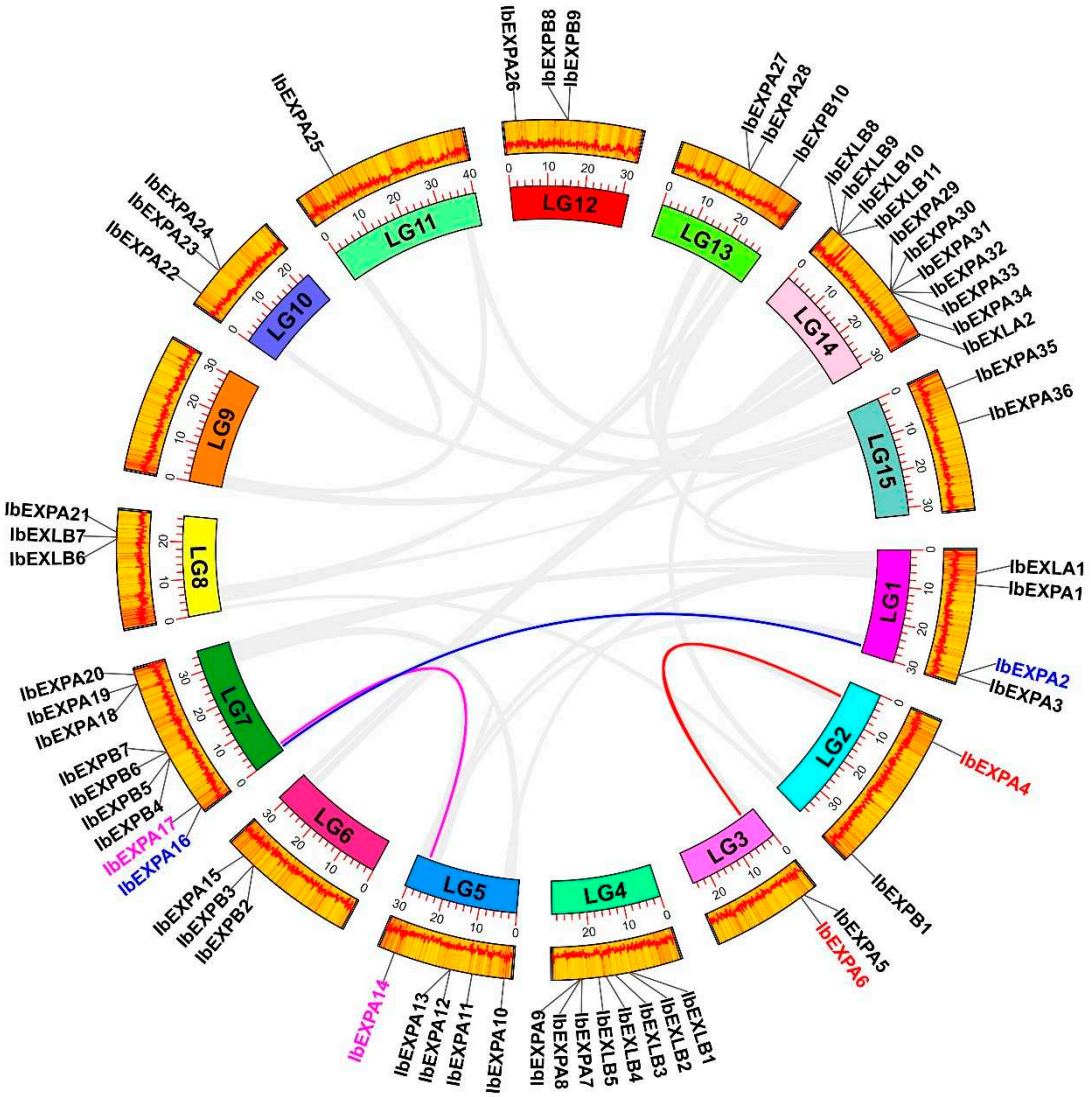

Figure 3. Segmental duplications and collinearity analysis of *IbEXPs* in sweetpotato. LG1- LG15 are represented by different colored rectangles. The heatmap and polyline along each rectangle depict the gene density of each chromosome. Duplicated IbEXP gene pairs on sweetpotato chromosomes are indicated by colored lines, and these corresponding genes are also marked with colors. Other *IbEXPs* genes which exhibit no collinear relationships were marked with black color.

2.5. Collinearity analysis of EXP genes between sweetpatato and other plants

To further investigate the origin and evolutionary relationships of sweetpatato *IbEXP* genes, collinearity relationships among *IbEXPs* and orthologous genes in nine representative plants was explored, including *Ipomoea triloba* (the likely diploid wild relative of sweetpotato), two cereal plants, two representative model plants, two Solanaceae plants, and two Brassica plants. The results exhibited that a total of 30 (50.8%) *IbEXPs* shared the collinear relationships with that in *Ipomoea triloba*, followed with *Solanum lycopersicum* (13), *Arabidopsis* (5), *Capsicum annuum* (4), *Brassica oleracea* (3), *Brassica rapa* (2), while there were no collinear relationships of *IbEXPs* with that in *Triticum aestivum*, *Oryza sativa* and *Zea mays* (Figure 4, Table S3-1/-2/-3). Therefore, the largest number of collinearity relationships exist between sweetpatato *IbEXPs* and *Ipomoea triloba* genes suggesting a closer relation between these two plants. Moreover, these data also showed that multiple *IbEXPs* had the collinear relationships with two genes in other plant species, particularly with *Ipomoea triloba*. Analogously, two *IbEXPs* genes shared the collinearity with the same one gene of detected four plants (*Ipomoea triloba*, *Arabidopsis thaliana*, *Brassica oleracea*, and *Solanum lycopersicum*) were also found

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(Table S3-1/-2/-3). These data indicated that a number of orthologous genes might originate from the common ancestor in these plants.

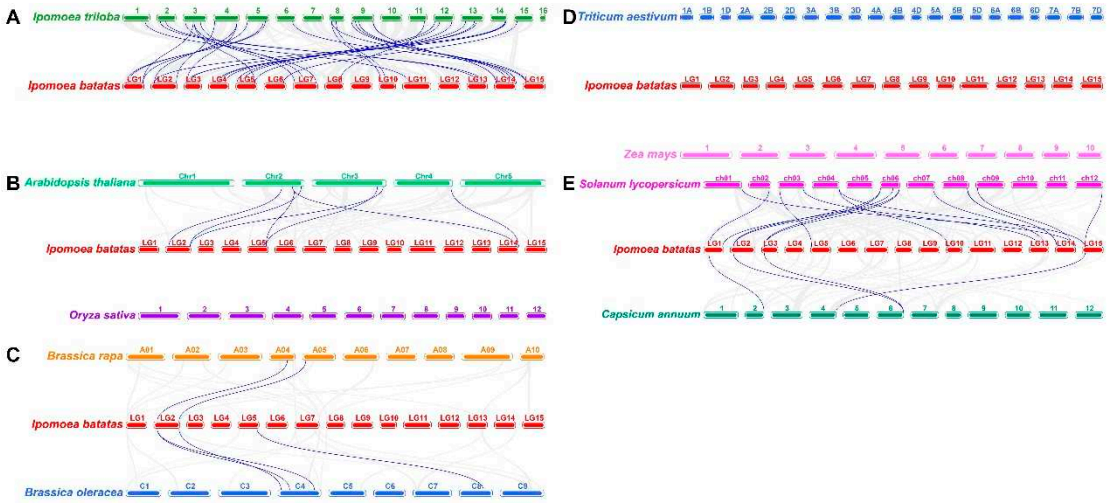

Figure 4. Synteny analyses of sweetpotato *IbEXPs* with other nine representative plants. These plants include *Ipomoea triloba* (A), *Oryza sativa* and *Arabidopsis thaliana* (B), *Brassica rapa* and *Brassica oleracea* (C), *Triticum aestivum* and *Zea mays* (D), *Solanum lycopersicum* and *Capsicum annuum* (E). The chromosomes of various plants are distinguished with differential colors.

2.6. Motif, conserved domain and gene structure analysis of IbEXPs

To further evaluate the sequence characteristics of sweetpatato IbEXPs, the investigation of conserved motif composition was performed by the MEME tool. The results exhibited that a total number of 20 distinct motifs were detected from IbEXPs proteins that are based upon the settings of *Arabidopsis* and rice [15]. The results showed that the IbEXPs belonging to the same subfamilies generally harbored the similar motif compositions, and these results further supported our subfamily classification (Figure 5 A-B). Motifs 2, 5 and 11 widely existed in the most IbEXPs, and other motifs distributed in certain IbEXP proteins. Universally, multiple motifs almost existed in all IbEXP members in the same subfamily and there were some composition differences of motifs among different subfamilies. For example, almost all members of EXPA, EXPB, EXLA, EXLB subfamilies harbored motifs 1, 2, 3, 4, 5, 6, 7, 8, 11, 20, motifs 2, 3, 5, 7, 10, 11, 16, motifs 2, 6, 9, 10, 11, 13, motifs 2, 5, 6, 11, 13, 15, respectively. And the results also displayed that some IbEXPs in the same subfamily contained specific motifs except the common motifs they had, such as motif 4, 8, 17 and 18 in EXPA, motif 14, 16 and 19 in EXPB. These results suggested that the compositions and number vary observably in these four subfamilies, and the existence of these specific motifs may suggest that sweetpatato IbEXPs had distinct and diverse functions.

To expore the sequence diversity of *IbEXPs* genes, the exon-intron compositions and conserved domain in each *IbEXPs* genes were examined. Conserved domain examination using Batch CD-Search showed that five domains with one pollen_allerg_1 domain and four typical EXP domains were examined from all IbEXPs (Figure 5 C). In the same subfamilies, most IbEXPs contain the same EXP domain and the pollen_allerg_1 domain, and the EXP domain located on the similar position with few exceptions. These data indicated that the EXP domain is the most valuable information construct distinctly the phylogenetic relationships among IbEXPs members. Gene structure detection displayed that the exon numbers of each *IbEXPs* gene varied from 1-14, with one *IbEXPs* genes containing no introns and four *IbEXPs* genes only having 1 intron (Figure 5 D). *IbEXPA19* harbored the most exon (14), following by *IbEXPA22* and *IbEXPB3* with 11 exons. Moreover, this result displayed that most of *IbEXPs* genes generally harbored similar gene structures and exhibited similar exon length. A certain difference of intron numbers among *IbEXPs* genes in the same subfamily were also found, which may associated with the function diversity of *IbEXPs* genes. All these results

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obtained here demonstrated that the phylogenetic relationships of IbEXPs were mainly related to their conserved EXP domains and gene structures.

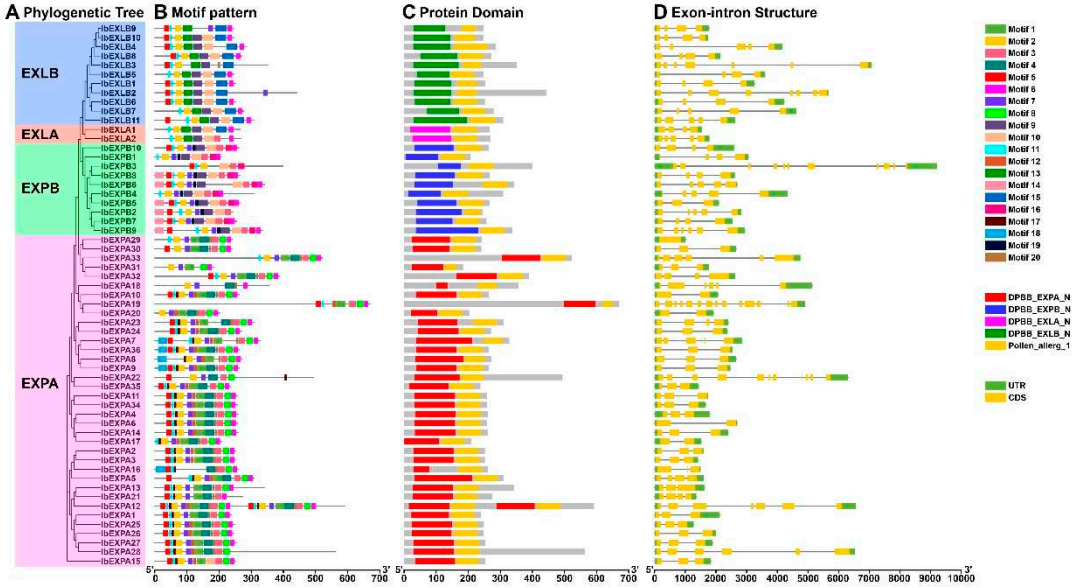

Figure 5. Phylogenetic tree, motif pattern, protein domain and gene structures of 59 sweetpotato *IbEXPs*. (A). The phylogenetic tree of 59 IbEXPs was constructed by MEGA 11.0 based on the consistent parameters used in Figure 1. (B). Distributions of motifs in each IbEXP protein. 20 motifs were identified by MEME data. (C). Conserved domain distributions of IbEXP proteins. The CD-search of NCBI database was used to detect the distributions of conserved domains of IbEXP proteins. The different colorful boxes present diverse conserved domains of each subfamily. (D). Gene structures of 59 sweetpotato *IbEXPs*. Green and yellow bars were used to represent the UTR and exons, respectively. The black lines were employed to indicate the introns. The length of IbEXP proteins or genes were estimated using the scale at the bottom.

2.7. Cis-element prediction in IbEXPs promoter regions

The cis-elements which locate in gene promoter region are the non-coding sequences. They are also vital for gene expression and widely regulate numerous biological processes [66]. To expore the possible regulatory mechanism of *IbEXPs* genes in controlling plant growth and response to stresses and hormones, the 2000bp sequences upstream of the start codon ATG of each *IbEXPs* gene was used to detect the cis-elements via the PlantCARE database. The results showed that a total of 742 cis-elements were found in the pormoter regions of *IbEXPs* genes (Table S4-1). All these cis-elements were associated with 19 types of biological processes (Figure 6 A, Table S4-2). Among them, *IbEXLB5* (g15300.t1) contained the largest number (31) of cis-elements. The cis-element numbers in each subfamily varies greatly (Figure 6 B) and all detected cis-elements in this study can be classified into three categories (Figure 6C) :

The first category relates to the hormone responses (404), including the salicylic acid responsive element (21, 2.83%), MeJA responsive element (90, 12.13%), gibberellin responsive element (34, 4.58%), ethylene responsive element (59, 7.95%), auxin responsive element (40, 5.39%), and abscisic acid responsive element (160, 21.56%). All subfamilies of *IbEXPs* genes abundantly contain the abscisic acid responsive elements (G-Box and ABRE), gibberellin responsive elements (GARE, P-box, TATC-box and CARE) and ethylene responsive element (ERE) in their promoter regions. Members in EXPA subfamily contained the maximum number of abscisic acid responsive elements, auxin responsive elements, MeJA responsive element, ethylene responsive elements and salicylic acid responsive elements compared with other three subfamilies, while members in EXLA harbored the smallest number of these five hormone related elements. It is worth noting that only one gibberellin responsive element were found in promoter region of EXLA members. Among these five hormone responsive element, the number (43 *IbEXPs* genes) of *IbEXPs* genes that contained the abscisic acid

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responsive element was the largest, followed by ethylene responsive element (34 *IbEXPs* genes). Besides, some *IbEXPs* genes, such as *IbEXPA2/8/9/17/23/24/26/34/36*, *IbEXPB3/4*, *IbEXLA1* and *IbEXLB6/10/11* had multiple common hormone responsive elements, manifesting the possibility that these members had more rapid intense and response to certain hormones. Simultaneously, *IbEXPA1/2/8/911/17/24/25/27/31/37/39*, *IbEXPB3/7/8/12* and *IbEXLB1/2/3/9/10* harbored diverse hormone responsive elements manifesting their potential roles in many networks of hormone regulation.

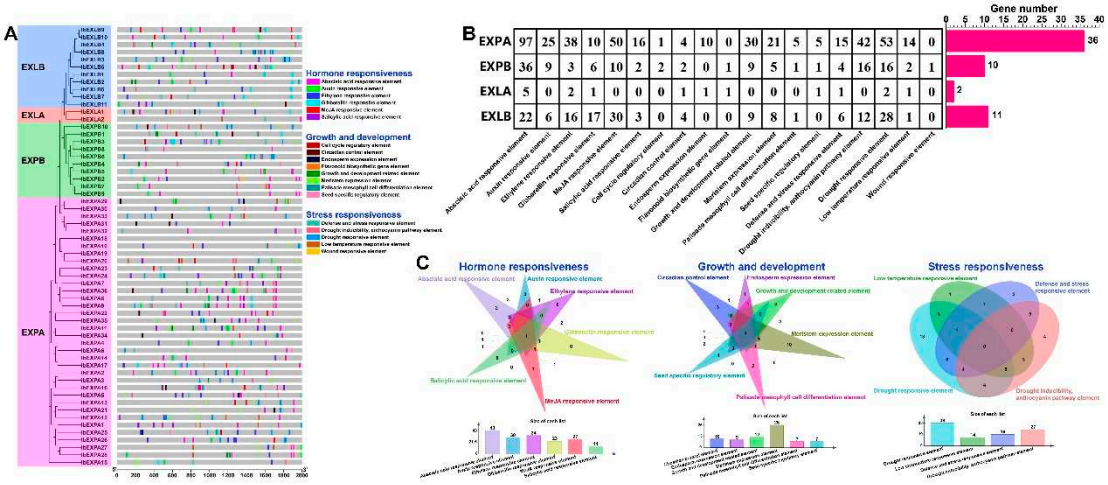

Figure 6. Predicted cis-elements in the promoters of 59 sweetpotato *IbEXPs*. (A). The phylogenetic tree and predicted cis-elements detected from 2000 bp promoter regions of each *IbEXPs* gene by PlantCARE database. The same phylogenetic tree as Figure 5 was used. All cis-elements are classified into three categories: hormone responsiveness, growth and development, and stress responsiveness. (B). The number of cis-elements in promoter of *IbEXPs*. The left table represents the number of each kind of cis-element found in each subfamily. The red rectangles indicate the gene number in each subfamily. (C). Venn diagram of three categories of cis-elements.

The second category relates to growth and development (123), including cell cycle regulatory element (3, 0.4%), circadian control element (11, 1.48%), endosperm expression element (11, 1.48%), flavonoid biosynthetic gene element (2, 0.26%), growth and development related element (48, 6.47%), meristem expression element (34, 4.58%), seed specific regulatory element (7, 0.94%), palisade mesophyll cell differentiation element (7, 0.94%). As shown in Figure 6B,D, some cis-elements were absent from certain subfamily, such as endosperm expression element, cell cycle regulatory element, flavonoid biosynthetic gene element, growth and development related element, meristem expression element and seed specific regulatory element. Cis-elements related to meristem expression had the largest number of *IbEXPs* (26), followed by growth and development related element (12 *IbEXPs* genes). The EXPA subfamily contained all these eight elements and had the maximum number of cis-elements that related to endosperm expression, growth and developmen and meristem expression compared with other three subfamilies. These results indicated that members of the EXPA subfamily may be the major regulator during plant growth and development.

The third category relates to stress responsiveness (214), including defense and stress responsive element (26, 3.5%), drought inducibility, anthocyanin pathway element (70, 9.43%), drought responsive element (99, 13.34%), low temperature responsive element (18, 2.43%) and wound responsive element (1, 0.13%). Similar to the two categories above, the EXPA subfamily also contained the largest number of cis-elements that related to drought inducibility, defense and stress responsive, anthocyanin pathway, drought responsive and low temperature responsive. The wound responsive element was absent from EXPA, EXLA and EXLB subfamily, and only one of this element existed in EXPB. Members in the EXPA subfamily contained the maximum number of drought responsive element, indicating that the EXPA members may be the major regulator in drought

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responsive. Moreover, the drought responsive element also had the largest number of *IbEXPs* genes, suggesting the major roles of of *IbEXPs* genes may mainly in drought responsive.

In short, the number and composition of cis-elements in promoter sequences of different *IbEXPs* genes exhibited great diversity in and among subfamilies. These results suggeseted that the gene expression levels of *IbEXPs* in sweetpotato are controled by diverse cis-elements in connection with growth and development, hormones and stress response.

2.8. Transcriptome-wide identification of IbEXPs genes associated with tuberous root development and their expression profiles in different tissues

Increasing evidence suggested that EXP genes played critical and various roles in different developmental processes, such as root growth and architecture, fruit softening and ripening, seed production, nodule formation and development. To investigate the potential biological roles of *IbEXPs* genes in tuberous root formation- and development, their transcript levels were explored in different developmental stages (FR, DR and MR) based on our previous transcriptome data [67]. We found that more than 20 of the 59 identified *IbEXPs* genes were significantly expressed in these three periods. To verify our transcriptome data, the expression patterns of eight selected *IbEXPs* genes which showed distinct expression changes in our RNA-seq data were analyzed in different periods of tuberous roots and other tissues by qRT-PCR assay. The results displayed that the transcript abundances of these *IbEXPs* genes showed varying degrees in different tissues (Figure 7). The expression levels of *IbEXPA4*, *IbEXPA17*, *IbEXPA25*, *IbEXPB10* and *IbEXLA1* were increased during tuberous root development (DR1, DR2, DR3 and MR), and were significantly higher in MR than other three stages (DR1, DR2 and DR3). Moreover, *IbEXPA17*, *IbEXPA25*, *IbEXPB5*, *IbEXLA1* and *IbEXLA2* were also highly expression in leaves. In the ealy stage DR1, the expression of *IbEXLA2* was lower, and then was dramatically increased at DR2. After that, its transcripts were induced with the development of tuberous root. The expression of *IbEXLB11* was down-regulated with the development of tuberous root development and was significantly accumulated in stems. The expression of *IbEXPB5* were increased following tuberous root development and showed the highest expression levels in DR3 and finally were reduced in MR. While the expression levels of *IbEXLA1* in stems and leaves and *IbEXLB11* in stems were significantly higher than that in different stages of tuberous roots. These results suggested that these selected EXP genes might play signifcant roles in different tissues or developmental stages of sweetpotato.

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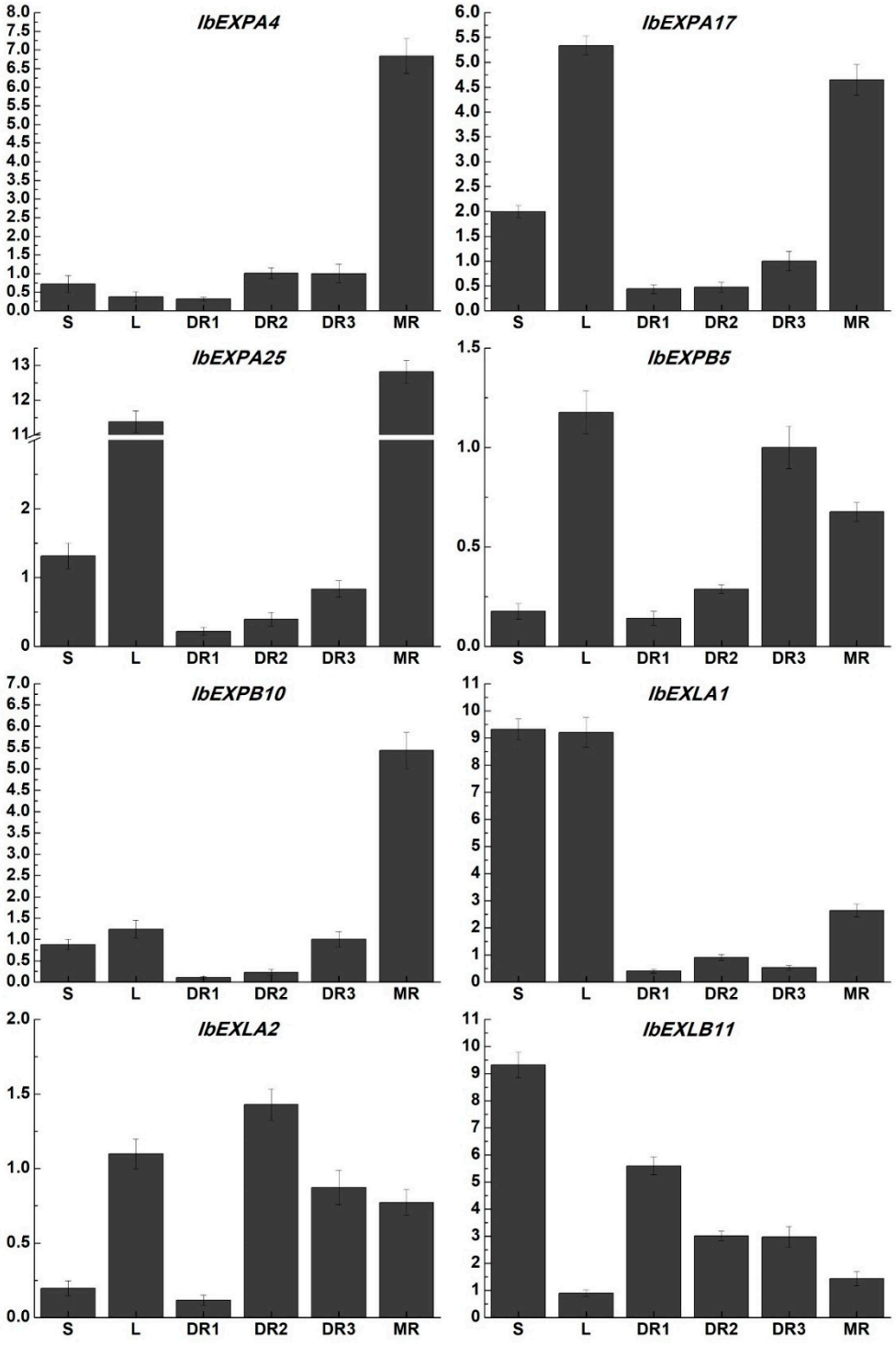

Figure 7. Expression profiles analysis of 8 *IbEXPs* in different tissues by qRT-PCR. L, mature leaves at 60 dap; S, stems at 60 dap; DR1, tuberous roots at 30 dap; DR2, tuberous roots at 60 dap; DR3, tuberous roots at 100 dap; MR, mature tuberous roots at 120 dap. Bars indicate the mean of three biological replicates ± SE.

2.9. Expression pattern analysisis of IbEXPs genes under multiple hormone treatment and abiotic stresses

In addition to the crucial functions in plant development, EXPs were also verified to participate in response to multiple abiotic stresses and exogenous plant hormones [27,37,68]. Thus, the transcript accumulation of selected eight *IbEXPs* genes under the treatments of stress and plant hormones were investigated. The results showed the expression levels of these *IbEXPs* were enhanced or decreased to varying degrees under different hormone treatments (Figure 8). Wherein, the transcriptional levels

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of *IbEXPA4/17/25*, *IbEXPB5/10*, *IbEXLA1*, *IbEXLB11* can be increased to diverse degrees by these four hormones (ACC, ABA, JA and SA). On the contrary, the expression levels of *IbEXLA2* were reduced under these four hormones. The transcripts of *IbEXPB5/10*, and *IbEXLA1* displayed higher fold changes with 6.6-7.9 fold compared to 0h data by ABA treatment, while other four *IbEXPs* genes (*IbEXPA4/17/25*, *IbEXLB11*) exhibited lower fold changes with less than 2 fold. The expression levels of *IbEXPA4/17/25*, *IbEXPB5* and *IbEXLB11* were up-regulated about 2.9-26.3 fold compared with that in 0h example under the ACC treatment. After being treated by JA, the transcript levels of *IbEXPA17/25*, *IbEXPB5*, *IbEXLA1* and *IbEXLB11* were induced by 2.9-13.8 fold at certain time points. While being treated by SA, only *IbEXLB11* displayed the increased 2 fold changes of transcript abundances, the transcripts of other seven EXP genes were decreased to varying degrees at all or some time points.

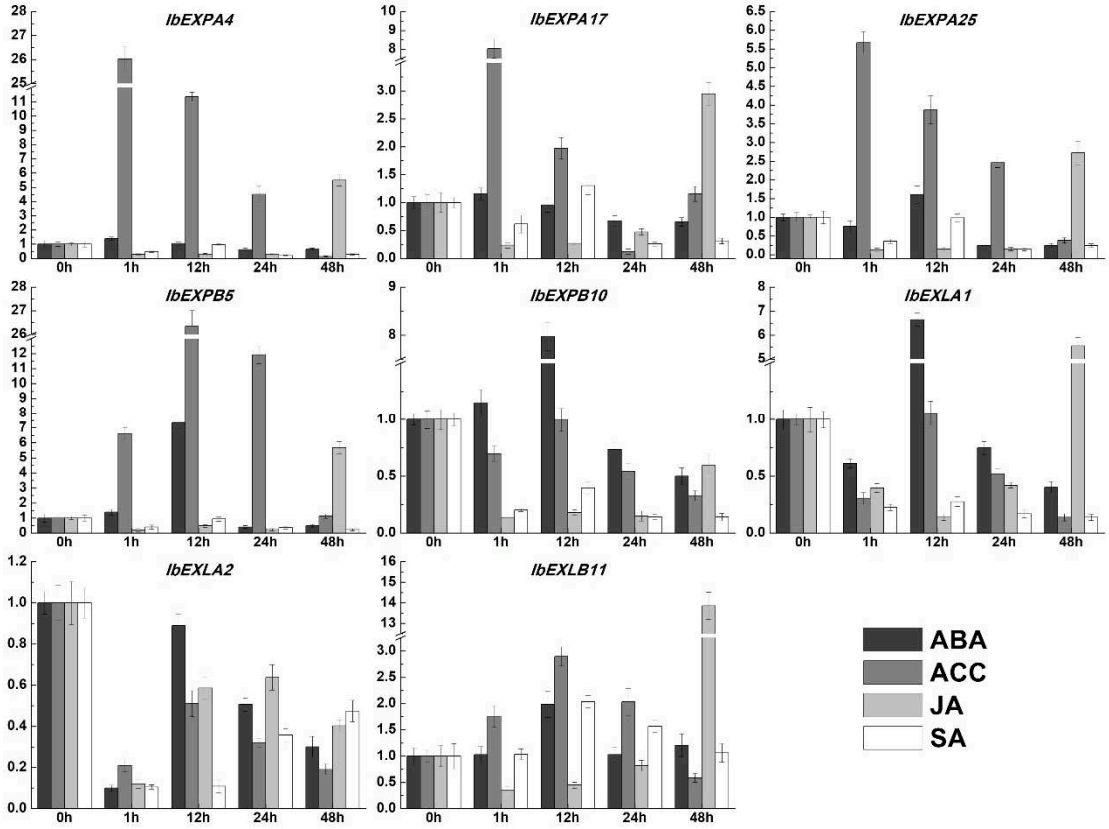

Figure 8. Relative expression levels of 8 *IbEXPs* detected by qRT-PCR under diverse hormone treatments. These hormone treatments include ABA(100 μM), ACC (100 μM), JA (100 μM) and SA (2mM). 0h represent the WT seedlings that were not treated by each treatment. Bars indicate the mean of three biological replicates ± SE. The two-fold expression changes of *IbEXPs* in each treated sample compared to 0h sample is considered to be the significant expression changes.

In consideration of the crucial functions of *IbEXPs* genes in various abiotic stresses reported in previous studies , we also performed the expression pattern analysis of selected *IbEXPs* genes under low and high temperature according to our previous study [69]. The results displayed that transcripts of selected eight *IbEXPs* increased or reduced to different degrees (Figure 9). The transcripts of *IbEXPA4/25*, *IbEXPB10*, *IbEXLA1* and *IbEXLA2* were significantly reduced at all time points. The transcripts of *IbEXPA17* were markedly reduced at all or some time points under low temperature stress, while its expression were increased about 2.3 fold at 48h. On the contrary, the expression levels of *IbEXPB5* were dramatically reduced at all or some time points under high temperature stress, but were observably increased about 4.1-9 fold at 24h and 48h. These results suggested that many *IbEXPs* genes may function as crucial regulators in response to some hormone (especially ABA, ACC and JA), multiple abiotic stresses and/or signal transduction.

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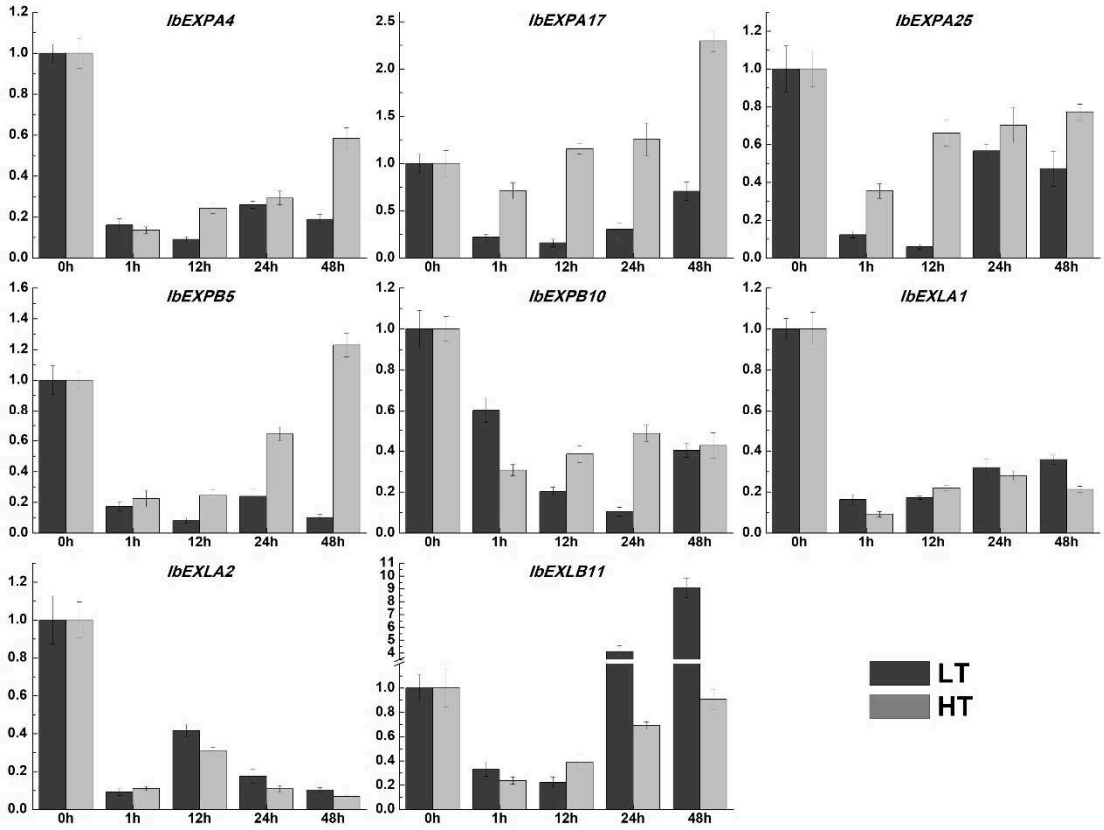

Figure 9. Expression profiles analysis of 8 *IbEXPs* under abiotic stresses by qRT-PCR. The abiotic stress treatments include low temperature (LT) and high temperature (HT). 0h represent the WT seedlings that were not treated by each treatment. Bars indicate the mean of three biological replicates ± SE. The two-fold expression changes of *IbEXPs* in each treated sample compared to 0h sample is considered to be the significant expression changes.

3. Discussion

Sweetpotato is a significant food crop and is broadly used in industrial materials, animal feed and human food. It has various natural advantages, such as strong stress resistance, high yield, wide adaptability [53]. Plant growth is determined by cell enlargement and proliferation and is restricted by cell wall, which limits the protoplasm increases of plant cells. The mechanism of cell wall extension has always been the focus of investigation due to the crucial functions of cell wall enlargement during plant morphogenesis [6]. Expansins are necessary for plant development and play critical roles in the relaxation of plant cell wall. As one of the most investigated structural proteins, expansins promote the growth of primary cell wall in plants. Expansins can loosen cell wall via cleaving hydrogen bonds between hemicellulose and cellulose microfibrils and can fill microfibrils and combine cellulose networks to form reticular systems [21,70], resulting in the increases of cell-wall strength and toughness and the continuous extension of cell wall [64]. However, members of expansin gene family in *Ipomoea batatas* are still not systematacially and comprehensively characterized. The completed genome sequencing of sweetpotato and advanced bioinformatics provide excellent foundations to perform the identification and characterisation of specific gene families [56]. In this study, the *IbEXPs* genes in *Ipomoea batatas* were systematically characterized and analyzed and their multifarious molecular characterizations were further implemented. Our study will provide lay the fundation for the further investigatins of regulatory modes and molecular functions of *IbEXPs* genes in sweetpotato growth and development and stress tolerance.

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3.1. Characterization of IbEXPs in sweetpotato

In this study, 59 IbEXPs genes were identified from *Ipomoea batatas* genome, while only 37 *ItfEXP* genes were identified in *Ipomoea trifida*, suggesting that the number expansion of EXP gene family has occurred in *Ipomoea batatas* compared with to its diploid wild relative [55]. These 59 IbEXPs genes were divided into four subfamilies, *IbEXPA*, *IbEXPB*, *IbEXLA* and *IbEXLB*, which were similar to other plant species. The number of 59 IbEXPs genes is more than 46, 38, 36, 46, 36 and 38 in barley [59], *Brachypodium distachyon* [60], *Arabidopsis* [15], gingkgo [62], potato [71] and tomato [72], and is similar to 52 and 58 in tobacco [64] and rice [15], while is obviously less than 75, 88, 92, 93 and 241 in soybean [61], maize [58], sugarcane [4], cotton [63] and common wheat [57], suggesting that member number of EXP genes has changed significantly in different plants during evolution. Likewise, the sizes of expansin subfamilies are unevenly distributed in different plant species and the EXPA subfamily occupies the highest proportion compared with other three subfamilies in alll plants (Table 2). In addition, 59 IbEXPs genes were disproportionately distributed on 14 chromosomes. Similar uneven distributions were also observed in wheat [57], cotton [63], barley [59] and potato [71] et al., indicating that the number of *IbEXPs* genes on each chromosome is irrelevant to the size of chromosomes. Therefore, *IbEXPs* genes on chromosomes were relatively clustered instead of averagely distributed, which may be due to the uneven gene replications of chromosome fragments.

Table 2. Summary of each expansin subfamily in 15 plant species.

Species	EXPA	EXPB	EXLA	EXLB	Total	Reference
Ipomoea batatas	36 (61%)	10 (17%)	2 (3.4%)	11 (18.6%)	59	In this study
Ipomoea trifida	23 (62.2%)	4 (10.8%)	2 (5.4%)	8 (21.6%)	37	[55]
sugarcane	51 (55.4%)	38 (41.3%)	3 (3.3%)	0 (0%)	92	[4]
Oryza sativa	34 (58.6%)	19(32.8%)	4 (6.9%)	1 (1.7%)	58	[15]
Triticum aestivum	121 (50.2%)	104 (43.2%)	16 (6.6%)	0 (0%)	241	[57]
maize	36 (40.9%)	48 (54.5%)	4 (4.5%)	0 (0%)	88	[58]
barley	24 (52.2%)	16 (34.8%)	6 (13%)	0 (0%)	46	[59]
Brachypodium distachyon	30 (79%)	4 (10.5%)	3 (7.9%)	1 (2.6%)	38	[60]
Arabidopsis	26 (72.2%)	6 (16.7%)	3 (8.3%)	1 (2.8%)	36	[15]
soybean	49 (65.3%)	9 (12%)	2 (2.7%)	15 (20%)	75	[61]
Ginkgo biloba	32 (69.5%)	4 (8.7%)	5 (10.9%)	5 (10.9)	46	[62]
cotton	67 (72%)	8 (8.6%)	6 (6.5%)	12 (12.9%)	93	[63]
tobacco	36 (69.2%)	6 (11.5%)	3 (5.8%)	7 (13.5%)	52	[64]
potato	24 (66.6%)	5 (13.9%)	1 (2.8%)	6 (16.7%)	36	[71]
tomato	25 (65.8%)	8 (21.1%)	1 (2.6)	4 (10.5%)	38	[72]

Although the IbEXPs protein properties diplayed significant differences, IbEXPs in the same clade harbored the relatively conserved gene structures, motifs and domains, which could provide an important reference for the phylogenetic analysis and functional investigation. These results verify that genes originated from ancestor can gradually expand and evolve [63]. IbEXPs members in the same subfamily tend to display similar compositions of motifs, domains and exon/intron structure, which were similar to that in other plant species such as tobacco, soybean, and cotton [61,63,64]. Gene structure exploration exhibited that about 83.1% of *IbEXPs* genes harbored 1-5 exons, which displayed the similarity with EXP genes in maize [58], soybean [61], tea [73], *Populus* [74] and banana [75]. However, several *IbEXPs* genes exhibited a distinct exception with over 8 exons, indicating the high divergences among them. The N-terminal conserved motifs (DPPB domains) of IbEXPs proteins

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which is rich in cysteine (Cys) residues with a characteristic catalytic domain may be related to the disulfide bond formation [13]. The C-terminal motif (pollen_allerg_1 domain) which cantains the conserved aromatic amino acids and polar tryptophan residues on its surface was considered as the polysaccharide binding domain. These motifs exist in IbEXPs proteins suggest that these motifs are crucial for the functions of IbEXPs in cell wall enlargement and loosening. Though the conserved DPBB and pollen_allerg_1 motifs are similar among most IbEXPs in the same subfamily, the IbEXPs showed significant differences of the molecular characteristics, which may be generated by the sequence differences in their non-conservative regions. Some specific motifs are only found in certain subfamilies, suggesting that some *IbEXPs* may play specific roles in plant development, because previous studies have widely reported that EXPs perform functions in various biological processes [17,26,34,37,41].

3.2. Evolutionary relationship and collinearity analysis of IbEXPs in sweetpotato

Phylogenetic relationship analysis showed that 59 IbEXPs were grouped into 4 subfamilies based on sequence homologies and subfamily classifications in *Arabidopsis* AtEXPs and rice OsEXPs [15,20,58]. At least one IbEXPs protein was found in each subfamily of rice and *Arabidopsis* [15,20], indicating that the discrepancies of EXP proteins may be earlier than dicots and monocots. The memebers of IbEXPs protiens in sweetpotato were unevenly distributed in the four subfamilies and most of them were the members of EXPA and EXPB subfamilies, while only a few of IbEXPs proteins blong to the EXLA and EXLB subfamilies. Similar uneven distribution events of members in these four subfamilies are also found in other plants, such as sugarcane [4], rice [15], *Arabidopsis* [15], cotton [63], potato [71] and tomato [72]. These results indicated that members of EXPA and EXPB subfamily may play more crucial roles in plant growth and development. For instance, the rice *OsEXPA10*, an Al-inducible expansin gene in rice, plays significant roles in cell elongation of roots [41]. Overexpression of *GhEXPA8* in cotton improves the length of fibers and micronaire value [76]. *TaEXPA2* plays crucial functions in seed production, and multiple abiotic stresses (salt, drought, oxidative and Cd) [27–30,77]. The β-expansin gene *OsEXPB2* participate in the architecture of root system in rice [37]. Moreover, the proportion of members in the same subfamily of different plants varies greatly. The classification of IbEXPs proteins displayed similarities and discrepancies compared to that in other plants, suggesting that more diversity of functions and structures exist in EXP proteins in different plants.

Gene duplications are vital driving forces during the processes of expansion and evolution of many gene families in plants, which can promote the generation of novel functional genes and species, and then plant species can better resist adverse environmental conditions in the process of evolution [65,78,79]. Previous investigations in moso bamboo [13], maize [58], barley [59], potato [71], banana [75], and *Brassica* species [79] suggest that the duplication events of tandem, segments and genome may explain *IbEXPs* expansion and evolution in sweetpotato. Analogously, collinearity analysis displayed that multiple *IbEXPs* were identified to have the duplications of tandems and segmentals, suggesting that some *IbEXPs* genes may be generated via gene duplications, further supporting the mechanism that brings about the expansion of *IbEXPs* genes. And the segmental and tandem duplications have similar contributions to the increase of *IbEXPs* genes. Additionally, the *IbEXPs* genes exhibiting segmental duplications and tandem repeats usually come from the same subfamily, and the *IbEXPs* gene pairs were mainly from EXPA, EXPB and EXLB subfamily, indicating that the expansions of *IbEXPs* genes in specific subfamilies may be beneficial for sweetpotato growth and development and adaptation to changing environmental conditions. These results are similar to those of *IbEXPs* genes in moso bamboo [13], maize [58], and potato [71], and so on, indicating the critical evolutionary functions of egmental and tandem duplications in gene expansions.

Furthermore, synteny analysis estimating the relations between *IbEXPs* genes and the counterparts of nine plants were studied, including *Ipomoea triloba*, *Oryza sativa*, *Arabidopsis*, *Zea mays*, *Triticum aestivum*, *Capsicum annuum*, *Solanum lycopersicum*, *Brassica oleracea* and *Brassica rapa*. Among them, the largest orthologous gene numbers between sweetpotato and *Ipomoea triloba* were identified, which further proved their close evolutionary relations, following by *Solanum lycopersicum*,

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Arabidopsis, *Capsicum annuum*, *Brassica oleracea* and *Brassica rapa*. These gene pairs of orthologous may come from the common ancestor of sweetpotato and its corresponding plant species. In addition, more complex relations such as multiple *Ipomoea trilobato*-single sweetpotato genes or multiple sweetpotato-single *Ipomoea trilobato* genes was also found, indicating that the orthologous genes might have vital functions in *IbEXPs* evolutions in sweetpotato. Howerover, there were no orthologous genes between sweetpotato and three gramineae plants (*Oryza sativa*, *Zea mays*, and *Triticum aestivum*) and some EXP subfamlies such as EXLB were lost in many plants [4,57–59], which probably due to the abundant chromosomal fusions and rearrangements took place in the genomes, and the selective gene loss seriously obscured the identification of synteny relations [80]. These findings may might have something to do with phylogenetic relations between sweetpotato and these nine plants. And large-scale duplications predate plant divergence and play vital functions in *IbEXPs* in the expansions of *IbEXPs* gene family.

3.3. Expression patterns and functional prediction of IbANCs in sweetpotato

EXP gene family have received increasing attentions on account of their wide participation in various biological processes of plant development and responses to diverse stresses. Numerous documents have manifested that EXP genes are excellent candidates for regulating growth and development and improving stress tolerance of crops via molecular breeding [20,36,68]. For example, *OsEXPA8*, *OsEXPA10* and *OsEXPB2* in rice [37,39,41], *GmEXPB2*, *GmEXLB1* and *GmINS1* in soybean [8,18,36,38], *TaEXPA2*, *TaEXPA8*, *TaEXPB23* in wheat [27,28,30,68,81] etc. have been proved their crucial functions in plant development and/or responses to various adverse environmental conditions. Up to now, the functions of sweetpotato *IbEXPs* genes in regulating stress resistance and plant development are still poorly understood. The expression profiles of genes are closely related to their biological functions, and the identification of gene expression can be conducive to accelerate the characterisation of gene functions. In this study, our results of RNA-seq and qRT-PCR showed that the examined *IbEXPs* genes exhibited obvious differential expression in different tissues and after various treatments of hormones and abiotic stresses, suggesting their diverse and critical functions of *IbEXPs* genes in plant development and stress response. For instance，the expression levels of multiple *IbEXPs* genes, particularly *IbEXPA25*, *IbEXPB5*, *IbEXLA2* and *IbEXLB11* showed obvious differential expression in different tissues, and *IbEXPA4, IbEXPB5* and *IbEXLB11* were dramatically induced or suppressed under different hormone and abiotic stresses, manifesting that they may play significant functions in sweetpotato growth and development and stress/hormone response, and/or help plants to reduce the damage caused by various stress.

In addition, the potential functions of *IbEXPs* genes in growth and development and stress/hormone response were further supported by cis-element and evolutionary relationship analysis. EXP genes in the close subfamilies may derive from the same gene/fragment duplication events, and functional investigations of EXPs have verified their conserved and/or similar roles in the same subfamily of plants [8,20,22,36,39,41]. Previous studies have manifested that *TaEXPA2* [27–30], *OsEXPA8* [39], *OsEXPA10* [41], *TaEXPA8* [81] and *AtEXPA1* [82,83] in EXPA subfamily, and *AtEXPB1* [84], *GmINS1* [8], *OsEXPB2* [37], *GmEXPB2* [36,38] and *TaEXPB23* [68] in EXPB subfamily, *AtEXLA2* in EXLB subfamily [19,85], *GmEXLB1* [18], *MdEXLB1* [34] and *BrEXLB1* [86] and in EXLB subfamily function as crucial participants in tissue growth and development and/or various stress responses. The phylogenetic analysis showed showed that the *IbEXPA1/-25/-26* genes was closed linked to *AtEXPA1/-10/-15*, *IbEXPB10* gene was closed linked to *AtEXPB1/-3* and *OsEXPB16/-17*, *IbEXLA1/2* genes were closed linked to *AtEXLA1/-2/-3*. And the expression levers of detected eight genes (*IbEXPA4/-17/25*, *IbEXPB5/-10*, *IbEXLA1/-2*, *IbEXLB11*) displayed tissue specificity in different tissues, and their transcripts were observably induced or suppressed under various hormone and stress treatments, implying that they might play vital roles in regulating the pathways of plant development and stress responses. Furthermore, IbEXP members in the same phylogenetic branch exhibited similar and discrepant expression profiles, suggesting the diversity of their potential functions. Therefore, development and/or stress response related IbEXPs which were grouped in the same

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subfamily were speculated to take part in controlling plant development and/or stress/hormone responses.

Previous investigations have showed that the phytohormones play significant roles in controling plant development and adaptation of various adverse environmental conditions [87,88]. Moreover, the cis-elements which lacated in gene promoter regions also function as crucial regulators in gene expression. In this study, numerous cis-elements in connection with hormones, growth and development, and different stresses including ABRE, ARE, ERE, TGACG-motif, CCGTCC-box, TGA-element, P-box, as-1, CAT-box, LTR, MSA-like and TATC-box and so on were detected in IbEXPs promoters, suggesting that they might function as the necessary participants in growth and development regulation, hormone and/or stress signaling. And the expression levels of examined *IbEXPs* genes (*IbEXPA4*/*-17*/*-25*, *IbEXPB5*/*-10*, *IbEXLA1*/*-2* and *IbEXLB11*) were markedly increased or reduced by one or some hormones and abiotic stress. These results are similar to previous investigations in moso bamboo [13], *Ipomoea trifida* [55], tobacco [64], *Brachypodium distachyon* [60] and banana [75]. However, the exact biological functions of sweetpotato *IbEXPs* genes remain to be studied. Therefore, investigating the involvements of *IbEXPs* genes in develpoment and stress regulation can provide valuable information to illustrate their potential functions in growth and development and stress tolerance.

4. Conclusion

In present study, 59 IbEXP*s* genes were systematically identified and characterized from sweetpotato genome and unevenly distributed on 14 of 15 chromosomes. Their phylogenetic classification, conserved motif and domain, collinearity relationship, gene structure, cis-elements detection, molecular characterization and chromosome localization were systematically investigated. Duplication events such as the tandem and segmental duplication are conducive to the expansion of sweetpotato IbEXP gene family, and synteny analysis of orthologous genes between sweetpotato and typical plant species provided significant insights about the phylogenetic characteristics of *IbEXPs* genes in sweetpotato. Moreover, the results of transcriptome and qRT-PCR displayed differential and various expression patterns of *IbEXPs* genes in different developmental tissues and divers stress and hormone treatments. Multiple tissue-specific expression and hormone/stress-induced *IbEXPs* genes may have very close relationships with the transcriptional regulation of sweetpotato development and stress responses. In conclusion, the present results can not only contribute to further understanding the complexity and importance of EXP gene family, but also lay the foundation for the comprehensive analysis of potential functions in regulating plant growth and development and stress tolerance.

5. Materials and Methods

5.1. Identification of IbEXPs genes in sweetpotato

The genome data and GFF annotations files of sweetpotato were obtained from the online database *Ipomoea* Genome Hub (http://sweetpotao.com) [56]. The protein sequences of EXP members of rice and *Arabidopsis* were downloaded from rice genome annotation database (http://rice.plantbiology.msu.edu/) and TAIR (https://www.arabidopsis.org/) according to previous study [15], respectively. Then the EXP protein sequences of *Arabidopsis* and rice were employed as the query sequences to carry out the BLASTP program againsting all of the *Ipomoea batatas* protein sequences to identify all the possible EXP members in sweetpotato via the TBtools software with E-value ≤ 1e-5, NumofHits of 500 and NumofAligns of 250 [89]. Subsequently, the Prosite database (https://prosite.expasy.org/), Pfam database (http://pfam.xfam.org/) and NCBI batch CD-search (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi) were used to confirm all obtained candidate EXP members and to exclude the protein sequences that lacked the EXP domain. Finally, a total of 59 non-redundant proteins sequences were identified as the putative EXP members and were further studied. Each IbEXPs sequence of nucleotide and protein can be found in Supplementary file S1.

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5.2. Sequence alignment and phylogenetic analysis of EXP proteins

For phylogenetic analysis, the reported 36 *Arabidopsis* AtEXP proteins, 58 rice OsEXP proteins [15] and 59 sweetpotato IbEXP proteins were used to perform the phylogenetic tree analysis. Finally, 153 EXP protein sequences were used to carry out the multiple sequence alignmen employing the ClustalW with default parameters. After that, the MEGA software (version 11.0) was used to construct the unrooted phylogenetic tree by the neighbor-joining bootstrap method. The detailed parameters were as follows: poisson model, pairwise deletion and 1,000 replicates. The *Arabidopsis* and rice EXP protein sequences were provided in Supplementary file S1.

5.3. Analysis of motif pattern, conserved domain and protein property of IbEXPs

The online MEME Suite (version 5.5.4, https://meme-suite.org/meme/tools/meme) and the Batch CD-Search database (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi) were employed to explore the motif and conserved domains (standard results) of each IbEXP protein, respectively. The ExPASy database (http://expasy.org/) were used to evaluate each IbEXP protein property, including the Mw (molecular weight) and pI (theoretical isoelectric point). The prediction of subcellular locations and phosphorylation sites of each IbEXP protein were also performed using the online datebase Plant-mPLoc (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/) and NetPhos 3.1 (https://services.healthtech.dtu.dk/service.php?NetPhos-3.1), respectively.

5.4. Chromosomal location and collinearity analysis of IbEXPs

The *IbEXPs* location on the chromosome of sweetpotato was investigated using the GFF annotation information obtained from the *Ipomoea* genome website (https://sweetpotao.com/). To preform the synteny analysis bewteen *IbEXPs* and genes in other plants, the genome and GFF annotation files of *Ipomoea batatas* and other nine representative plant species (*Ipomoea triloba*, rice, *Arabidopsis*, maize, wheat, *Brassica rapa*, pepper, *Brassica oleracea* and tomato) were downloaded from TAIR, *Ipomoea Genome* Hub, Sol Genomics Network, EnsemblPlants database (http://plants.ensembl.org/index.html) and phytozome database (https://phytozome-next.jgi.doe.gov/). The collinearity relationships and gene duplications were investigated using Multiple Collinearity Scan toolkit (MCScanX) by a default parameter [90]. Then the circos and TBtools softwares with the 30 value as the minimum block size were employed to visualize these results [89,91].

5.5. Plant materials and treatments of abiotic stresses and hormones

To be consistent with the plant materials used in the RNA-seq in our previous study [67], the Taizhong 6 sweetpotato (*Ipomoea batatas*) plants were cultivated in a greenhouse in Jiangsu Normal University. Then the developing tuberous roots at 30 dap (DR1), 60 dap (DR2) and 100 dap (DR3)), mature tuberous roots at 120 dap (MR), mature leaves at 60 dap (L) and stems at 60 dap (S) were collected to analysis the expression patterns of selected *IbEXPs* genes. For the treatments of different hormones and abiotic stresses, plant seedlings of *Ipomoea batatas* (XuShu 22) harboring 3-4 mature leaves were cultured in a greenhouse. After a week of cultivation in water, the consistent seedlings were selected for the hormone treatments and abiotic stress. For hormone treatment, the seedlings were cultivated in water that contained 100 μM ACC, 100 μM ABA, 100 μM JA and 2mM SA, respectively. For abiotic stresses, seedlings were cultivated at 4 ˚C and 42 ˚C to simulate the cold and heat stresses, respectively. All needed leaf samples of controls and treated plants were harvested at 0, 1, 12, 24 and 48 h after various treatments. At least three independent biological replicates were collected for each treatment.

5.6. RNA extraction and qRT-PCR analysis

Total RNA in each collected sample was extracted using the RNA Extraction Kits (OMEGA, USA) following the method provided by manufacturers. The first-strand cDNA was synthesized via reverse transcription using 800 ng total RNA following the same methods in our study [92]. Then the

synthesized cDNAs was diluted by the RNase/DNase-free water. The qRT-PCR experiments according to the same the same method were implemented on the CFX96™ Real-Time System (Bio-Rad, USA) as described before [92]. The sweetpotato *IbARF* (accession number: JX177359) gene was applied as the reference gene [93]. All used primers in qRT-PCR can be found in Table S5. Three independent technical and biological repetitions were performed for each sample in the qRT-PCR.

5.7. Statistical analyses

Data were presented as mean ± SE standard deviation. A cut-off two-fold value for differential gene expression which was regarded as the biological significance was adopted [94]. The originPro software (v8.0, SAS Institute) were graphs were employed to visualize the results of qRT-PCR experiments.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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