Genome-wide in silico identification and expression analysis of beta-galactosidase family members in sweetpotato [*Ipomoea batatas* (L.) Lam.]

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Abstract

Background: Sweetpotato (*Ipomoea batatas* (L.) Lam.) serves as an important food source for human beings. β-galactosidase (bgal) is a glycosyl hydrolase involved in cell wall modification, which plays essential roles in plant development and environmental stress adaptation. However, the function of *bgal*s genes in sweetpotato has yet to be reported.

Results: In this study, 17 β-galactosidase genes (*Ibbgal*) were identified in sweetpotato, which were classified into seven subfamilies using interspecific phylogenetic and comparative analyses. The promoter regions of *Ibbgal*s harbored several stress, hormone and light responsive cis-acting elements. Quantitative real-time PCR results displayed that *Ibbgal* genes had the distinct expression patterns across different tissues and varieties. Moreover, the expression profiles under various hormonal treatments, abiotic and biotic stresses were highly divergent in leaves and root.

Conclusions: These findings suggest that *Ibbgals* may involve in plant development and stress responses through regulating the metabolism of cell wall polysaccharides.

Background

β-galactosidases (EC 3.2.1.23; bgal) widely exist in higher plants. Plant β-galactosidase belongs to the glycoside hydrolase 35 (GH35) families [1], which catalyzes the removal of terminal galactosyl residues from carbohydrates, glycoproteins and galactolipids [2, 3]. In plants, β-galactosidase has been reported to degrade structural polysaccharides in plant cell walls to release free galactose during a variety of biological processes, including cell wall expansion and degradation, metabolic recycling of galactolipids and glycoproteins, and turnover of signaling molecules during ripening [4, 5].

In higher plants, bgals have been grouped into two classes based on their substrate preference [6]. Enzymes in the first class prefer pectic β-(1→4)-galactan as the substrate, and enzymes in the other prefer the β-(1→3) and (1→6)-galactan backbones of arabinogalactan proteins [7, 8]. A typical bgall protein contains one conserved active site, which is a GH35 domain in the N-terminal region [9]. Like other glycosidase families, GH35 is ubiquitously expressed in many plants, such as tomato [2], papaya [10], *Arabidopsis* [11], *Brassica campestris* [12] and rice [13].

Plant *bgal* genes are widely involved in the modification of the architecture of cell walls and intercellular attachments [14, 15]. *bgal* genes also respond to plant growth and development including fruit development and ripening [16, 17], seed germination [18, 19], and root development [20, 21]. In most fruits, *bgal* genes exhibit differential expression patterns during flower and fruit development [16, 12]. In *Cicer arietinum*, the expression of *Canbgal-5* is related to early and meristematic stages with a high cell division rate, such as the meristematic hook, and apical internodes [3]. In addition, *bgal* genes can be regulated by abiotic and biotic stresses [22]. For example, *Atbgal1* was reported to be induced by salt stress or pathogen attack [23]. Likewise, the transcription level of β-galactosidase in cowpea is reduced under salt treatments [24], and the *bgal* gene in peach is highly suppressed by water stress [25]. In addition, *bgal* genes have been found to function in a variety of biological processes through ethylene signal transduction [26, 11]. However, the function of *bgals* in sweetpotato (*Ipomoea batatas* (L.) Lam) has yet to be fully understood.

Sweetpotato is an important food crop which is widely grown in tropical and subtropical areas, especially in Asia and sub-Saharan Africa. Due to its outcrossing hexaploidy (2n=6×=90), the genomic research in sweetpotato is very

complicated [27, 28]. So far, no high-quality genome sequence of sweetpotato has been available. Although *β-gal* genes are widely isolated in many plant species, its function in sweetpotato is largely unknown. In the present study, we identified 17 sweetpotato *bgal* genes (*Ibbgals*), and their sequences, motif compositions and promoters were investigated. The phylogenetic relationships of *bgal* genes in sweetpotato with those in *Arabidopsis* were explored. In addition, the expression patterns of these 17 *Ibbgals* in different tissues of two cultivars were investigated under three exogenous hormones, two abiotic and one biotic stress conditions. Our study will serve as a basis for further functional study of *bgal* genes in plants, and provide new insight into different regulatory mechanisms in plant growth through *bgal*-mediated responses to environmental stresses in sweetpotato.

Results

Identification and characterization of *Ibbgals* genes in sweetpotato

The conserved bgal domain in the N-terminal of each transcript was confirmed in the NCBI conserved domain database. A total of 17 *Ibbgal* genes were identified in sweetpotato. The deduced amino acid sequences of the *Ibbgal* proteins were used to predict their protein lengths, signal peptides, pI values, and molecular weights. The candidate bgal transcripts were identified using BLAST in two transcriptome databases (SRP068179 and CRA000288). The sub-cellular localization and possible N-glycosylation sites are summarized in Table 1. Characteristic analysis showed that these 17 Ibbgal proteins were 673 to 1110 aa in length, the predicted MWs and pIs ranged from 74.8 kDa to 125.1 kDa and 5.31 to 6.16, respectively. Eight, six and two Ibbgals were found to be in the chloroplast, vacuole, and nucleus, respectively. Only one Ibbgal, Ibbgal7, was found to be located in the extracellular. Signal peptides were found in the majority of these Ibbgals, except for Ibbgal4, Ibbgal5, Ibbgal10, Ibbgal13 and Ibbgal17. The number of N-glycosylation sites varied from 1 to 6, wherein both Ibbgal13 and Ibbgal16 contained 6 N-glycosylation sites.

Conserved motifs and phylogenetic analysis of the Ibbgal proteins

The β-galactosidase active site was found in all Ibbgal proteins. However, all but Ibbgal13 have the active site consensus sequence GGP[LIVM]xQxENE[FY] of the GH35 β-galactosidase family. Most Ibbgal members carried a Gal-lectin domain at the C-terminus of the protein sequence, except for Ibbgal2, Ibbgal5, Ibbgal12, Ibbgal13, and Ibbgal17. Motif analysis using MEME server showed that motif 1 was found in all Ibbgals except Ibbgal13, and motifs 2-6 were found in all except Ibbgal11 and Ibbgal17 (Fig. 1).

A total of 34 *bgal* genes from sweetpotato and *Arabidopsis* were classified into seven subgroups, designated as A, B, C, D, E, F and G, using phylogenetic analysis (Fig. 2). Among these *groups*, groups A and D were the largest groups with four *Ibbgal* genes in each. Groups B and E had three *Ibbgal* genes. *Ibbgal9*, *Ibbgal17* and *Ibbgal13* were classified into group C, F and E, respectively.

Cis-element prediction of *Ibbgal* genes

To understand the potential transcriptional regulatory mechanisms of the *Ibbgal* genes, the cis-elements of each *Ibbgal* promoter sequences were predicted and analyzed. As shown in Table 2, the promoters of the *Ibbgal* gene have at least four types of cis-elements, including plant hormone responsive elements, light responsive elements, stress responsive elements, and other elements. Most of the *Ibbgal* promoters had the GARE (gibberellin-responsive element), ABRE (Abscisic acid response element) and ERE (ethylene-responsive element), suggesting that *Ibbgal*s are involved in the regulation of plant growth and development as well as abiotic stress adaption. Likewise, auxin-

responsive region core sequences (AuxRE and CATATGGMSAUR) were found in most *Ibbgal* genes except *Ibbgal3*, *Ibbgal5*, *Ibbgal6*, *Ibbgal9*, *Ibbgal12*, *Ibbgal13* and *Ibbgal15*. At least five light response elements were found in each *Ibbgal* gene, which might be essential for plant growth and development and responses to various stresses. Most *Ibbgal* promoters contain circadian and EE elements except *Ibbgal6*, *Ibbgal16* and *Ibbgal17* that are participated in circadian regulation. Interestingly, all the *Ibbgal* sequences contain the MYC-like cis-element, which mediates the responses to dehydration, freeze and ABA stresses.

Expression profiles of *Ibbgal* genes in tissues and different root development stages

To identify the potential functions of *Ibbgal* genes, we analyzed the transcript levels of *Ibbgal* genes in different developmental stages of roots and different tissues including leaf, stem lip, stem, fibrous root, and storage root. As shown in Fig. 3A, 47% of the *Ibbgal* genes had similar expression patterns in five tissues from *cv*. Jishu25 and Jishu29. For example, *Ibbgal4*, *Ibbgal10*, *Ibbgal13* and *Ibbgal17* were highly expressed in five tissues, whereas *Ibbgal14*, *Ibbgal15* and *Ibbgal16* were poorly expressed in these tissues. Intriguingly, the expression of *Ibbgal4* in fibrous root was significantly higher than that of storage root, while *Ibbgal3* and *Ibbgal10* were expressed at higher levels in lip than other tissues. In addition, the transcript of *Ibbgal17* mRNA in *cv*. Jishu25 was prominently higher in storage root than fibrous root, whereas that in *cv*. Jishu29 had no significant difference between storage and fibrous roots. Similarly, the expression of *Ibbgal11* had the opposite pattern in the storage and fibrous roots between *cv*. Jishu25 and Jishu29.

In root development stages, 6 (35.3%) *Ibbgal* transcripts were down-regulated including *Ibbgal2*, *Ibbgal3*, *Ibbgal4*, *Ibbgal6*, *Ibbgal10*, and *Ibbgal16*, whereas 6 *Ibbgal* transcripts were up-regulated. Two *Ibbgal* genes (*Ibbgal14* and *Ibbgal15*) were not detected in root development. It is interesting that the *Ibbgal11* and *Ibbgal12* transcripts had the opposite expression pattern between *cv.* Jishu25 and Jishu29 (Fig. 3B). The up-regulated genes reached the peak expression during 55-95 days after transplanting.

Expression profiles of *Ibbgal* genes in response to abiotic and biotic stresses

Besides their functions in plant growth and development, *Ibbgal* genes may also be involved in biotic and abiotic stress responses. For the crop of sweetpotato, salinity and drought are the most dominant factors which limit the growth and yield among various abiotic stresses.Under salt stress, all *Ibbgal* genes were up-regulated in these two cultivars (Fig.4). Some genes had the highest expression levels at 12 h in the leaves of these two cultivars, whereas other *Ibbgal* genes in roots were expressed at a high level at 6 h and 48 h after salt stress. In addition, *Ibbgal2*, *Ibbgal4*, *Ibbgal5* and *Ibbgal13* in leaves of these two cultivars were unregulated remarkably by at least 10-fold induction after salt stress. Under drought stress (Fig. 4), all *Ibbgal* genes were up-regulated in the leaves and roots of *cv*. Jishu29, while *Ibbgal3*, *Ibbgal6*, *Ibbgal10*, and *Ibbgal17* were down-regulated in the leaves of Jishu25. *Ibbgal1*, *Ibbgal3* and *Ibbgal16* expression were also reduced in the root of Jishu25. Amongst the up-regulated genes, the expression of *Ibbgal2, Ibbgal4, Ibbgal8, Ibbgal9* and *Ibbgal13* reached the peak at 12 h after stress, and *Ibbgal4* was the most up-regulated gene with at least 81-fold induction in the leaves of these two cultivars. Black spot, caused by *Ceratocystis fimbriata*(*C. fimbriata*), is one of the main diseases in sweetpotato production, which seriously affects the quality and yield of sweetpotato. After pathogen infection, *Ibbgal* genes had different expression patterns in the leaves and roots of these two cultivars (Fig. 4). *Ibbgal5, Ibbgal10*, *Ibbgal11* and *Ibbgal16* transcripts were induced by pathogen infection in these two cultivars. It is worth noting that *Ibbgal15* expression in the leaves and roots of *cv.* Jishu25 was up-regulated, whereas down-regulated in *cv.* Jishu29 after the infection.

Collectively, the distinct expression pattern of *Ibbgals* in response to salt, drought and pathogen infection suggested that sweetpotato might have different regulatory mechanisms in response to different stresses.

Expression profiles of *Ibbgal* genes in response to various hormone treatments

bgal plays an important role in plant growth and development. Thus, it is necessary to survey the expression profiles of *Ibbgals* under various hormone treatments. After the uniconazole treatment, the expression of eight *Ibbgal* genes (including *Ibbgal3*, *Ibbgal6*, *Ibbgal9-12*, *Ibbgal16* and *Ibbgal17*) was induced to varying degrees in the leaves and roots of these two cultivars (Fig. 5). Among these *Ibbgals*, *Ibbgal6* was most significantly up-regulated with a 19.5-fold induction, whereas *Ibbgal3* was the least up-regulated with a 1.4-fold induction. Interestingly, *Ibbgal4* and *Ibbgal8* expression were up-regulated in *cv.* Jishu25, whereas down-regulated in cv. Jishu29 by the uniconazole treatment. The accumulation of four *Ibbgal* genes (including *Ibbgal4*, *Ibbgal6, Ibbgal11*, and *Ibbgal12*) were unregulated, while *Ibbgal5* was down-regulated by the GA_3 treatment (Fig. 5). Among these *Ibbgals*, *Ibbgal4* was the most up-regulated gene with a 59.4-fold induction, whereas *Ibbgal12* was the least up-regulated with a 1.9-fold induction. In addition, GA_3 treatments increased the expression of *Ibbgal5* and *Ibbgal10* in *cv.* Jishu29, but decreased the expression in *cv.* Jishu25. Moreover, the *Ibbgal* expression in cv. Jishu29 was more stable compare with that in *cv.* Jishu25 after the GA_3 treatment. For the ABA treatment, all *Ibbgal* transcripts were induced in the leaves of these two cultivars (Fig. 5). Among them, *Ibbgal4* was the most up-regulated gene with a 43-fold and 104-fold induction at 12 h after the ABA treatment. In the root of these two cultivars, most *Ibbgal* transcripts were up-regulated, whereas *Ibbgal1* and *Ibbgal15* were down-regulated under the stress. Among the up-regulated genes, *Ibbgal2* expression reached the peak with a 4.3-6.3 fold induction at 24 h. *Ibbgal4* was significantly induced in *cv*. Jishu25, while it was only slightly up-regulated in *cv*. Jishu29 with a 1.95-fold induction.

Discussion

Plant β-galactosidase participates in cell wall biogenesis and modification during plant growth [15, 17]. In this study, 17 β-galactosidase cDNAs were isolated from sweetpotato, which have the same number of β-galactosidases as in Arabidopsis, tomato and peach [29, 17]. All bgals except *Ibbgal13* had the active site consensus sequences GGP[LIVM]xQxENE[FY]. Most Ibbgal members contained a Gal-lectin domain at the C-terminus, which might be responsible for substrate specificity of bgals [11, 29]. In addition, most Ibbgals were predicted to have signal peptides in the N-terminus, which might be involved in cell wall-related biological processes [29]. A phylogenetic tree was constructed using the bgal proteins from sweetpotato, *Arabidopsis* and rice, which is similar to that observed in *Arabidopsis*, tomato and rice [29, 13]. This result implied that the bgals in the same branch might have similar and distinct functions, and bgal diversification might occur in the early stage of plant evolution. *Ibbgal4* and *Atbgal1* of groups A shared the same clade, suggesting that they have similar functions.

In a previous study, Esteban *et al*. (2005) found that *bgal* genes participate in the development of vegetative organs in *Cicer arietinum* [3]. *Atbgal* genes were reported to have differential tissue-specific expression patterns [11]. Similarly, the expression patterns of *Ibbgal*s were distinct in different tissues of sweetpotato in this study. Most *Ibbgal* genes were expressed in all tissues, whereas *Ibbgal14*, *Ibbgal15* and *Ibbgal16* had low expression levels in five tissues. The results are consistent with the observations in Arabidopsis reported by Gantulga *et al*. [30]. A number of cis-elements related to development, such as GCN4_motif, TATA box and RY-element, were found in the promoter of *Ibbgal* genes [31, 32], suggesting that these genes might be related to the development of sweetpotato. *Ibbgal2-4*, *Ibbgal6*, *Ibbgal10*, *Ibbgal12* and *Ibbgal17* were highly expressed in the early stages of root development. Previous reports have shown that *Atbgal5* is involved in root elongation through modifying the cell wall [21, 33].

Lovas *et al*. found that *Stubgal83* might participate in root and tuber development by altering the metabolic sugar status of the leaves [34]. Thus, we deduced that *Ibbgal*s might be associated with root development by modifying the cell wall and carbohydrate metabolism. Further study is needed to investigate the function of *Ibbgal*s during root development in sweetpotato.

To date, increasing evidence manifests that *bgal* genes are involved in response to various hormonal, biotic and abiotic stresses. *PaGAL3* and *PaGAL4* expression in avocado fruit were found to be inhibited by ethylene and ripening signals [26]. In plant coleoptile tissues, auxin-induced increase of elongation rates is closely associated with the β-galactosidase activity [35, 3]. Li *et al.* showed that the β-galactosidase genes in calamander are down-regulated through IAA, ethylene and JA after infection by fungus C*. acutatum* of citrus flower [36]. Our study shows that the upstream region of all *Ibbgal*s contained three to seven cis-elements related to phytohormone responses, such as GARE, ERE, AuxRE, CATATGGMSAUR. GARE and PYRIMIDINEBOXHVEPB1 are involved in GA_3 responses [37, 38]. In this study, the expression of eight *Ibbgal* genes was significantly up-regulated by the uniconazole treatment. Meanwhile, the majority of the *Ibbgal* genes were regulated by the GA_3 treatment in leaves and stems of these two cultivars. ABA is a requisite factor in response to stress, senescence, and fruit development [39, 40]. Spadoni *et al*. found that the expression levels of *bgal* genes decrease in peach fruit after hot water treatment [25]. Several *bgal* genes are regulated by abiotic and biotic stresses in *A. thaliana* and *Brassica campestris* [23, 12, 41]. In addition, the cis-elements related to stress responses, such as MYC-like, LRT, W-BOX, MBS and ACGT-motif, have been found in the promoter region of *ibbgal* genes, which might regulate gene expression during biotic and abiotic stresses [42, 43]. Similarly, our result showed that most *Ibbgal* transcripts were related to salt stress, drought stress, ABA treatment and pathogen infection. For example, the expression of all *Ibbgal4* was greatly up-regulated by salt and ABA treatments in the leaves of sweetpotato. Taken together, these *Ibbgal* genes play essential functions in biotic and abiotic stress responses and related signal transduction pathways.

In particular, almost all *Ibbgal*s exhibited different stress and hormone response patterns between leaves and roots, and have distinct expression levels between these two cultivars. There are different in pectin content from sweetpotato cultivars. β-galactosidase functions in the degradation of galactan side chains of pectin leading to cell wall loosening and softening [44, 45], suggesting β-galactosidase may be involved in the regulation of the pectin content, and different bgal-mediated pathways were activated in the storage root development. In respond to stresses, the sugars accumulated are reported to involve in osmotic adjustments to sustain cell structure and photosynthesis in plant [46, 47]. Pandy *et al*. [48] found that loss of sugar is the key regulator for activation of the cell wall hydrolase during senescence. β-galactosidase under abiotic and biotic stresses might be induce the initial structural modification of cell wall and activated to degrade cell wall polysaccharides for producing sugar. So, *Ibbgal* genes are mainly upregulated expressed under abiotic and biotic stresses. Further studies are needed to investigate the functions of different bgals in the stress-response system in sweetpotato.

Conclusion

We characterized 17 *Ibbgal* genes and then analyzed their motif compositions and N-glycosylation site. Based on the phylogenetic analysis, the bgals were divided into six subgroups. We also investigated their promoter regions and sub-cellular location. In addition, we systematically investigated the expression profiles in different tissues, and different development stages of storage roots, as well as the expression of the bgals under six different environmental treatments. The diversification of the bgal genes provides a solid foundation for further elaborating the bgal-mediated stress-response system in sweetpotato.

Methods

Identification and isolation of *Ibbgals* in sweetpotato

To identify *Ibbgal* genes, we performed local BLAST and domain search for genes containing the conserved domain of bgals in two transcriptase databases (SRP068179 and CRA000288). The obtained transcript sequences were translated and analyzed by the PFAM program (http://pfam.xfam.org) to examine the presence of the bgal conserved domains. The transcripts encoding short proteins with less than 120 amino acids were removed. The presence of the bgal domain was confirmed by analyzing the deduced proteins of the filtered transcripts in the NCBI BLAST. If two or more transcripts had the identity of amino acids equal to or higher than 97%, only one of these transcripts was kept in the final list of the genes. Pooled samples including 9 tissues of shoot, leaf, stem, fibrous root, tuberous toot, flower, salt-treated, drought-treated and ABA-treated plants were collected from two sweetpotato cultivars (Jishu25 and Jishu29). The total RNA was isolated from the pooled sample using TRIzol, and cDNA was synthesized using a reverse transcription Kit (Trasgene, China). To isolate the *bgal* genes, the gene-specific primers were designed used for PCR amplification (Additional file 1:Table S1). The obtained sequences were compared to the corresponding transcripts, and the related protein data are summarized in Table 1.

Protein properties, N-glycosylation site and subcellular location of the *Ibbgal* proteins

The molecular weights (MW) and isoelectric points (pI) of *Ibbgal* genes were analyzed using the ExPasy server (http://web.expasy.org/protparam/). N-glycosylation site analysis of *Ibbgals* genes was conducted using the NetNGlyc 1.0 server (http://www.cbs.dtu.dk/services/NetNGlyc/)[12]. The WoLF PSORT tools (https://wolfpsort.hgc.jp/) were used to predict the subcellular location of the bgal proteins [49].

Conserved motifs, phylogenetic analysis and promoter region prediction of the Ibbgal proteins

The conserved domains were identified by the online program SMART (http://smart.embl-heidelberg.de/). These 17 Ibbgal protein sequences were aligned with the MEME server (http://meme-suite.org/tools/meme). The protein sequences of the identified sweetpotato *bgal*s were aligned, and the phylogenetic tree was constructed using the Neighbor-Joining (NJ) method of MEGA software 7.0. The sequences of the bgal proteins from different species, including *Arabidopsis* [29], were obtained based on the description in the literature or downloaded from the plantgdb database (http://www.plantgdb.org/). The promoter sequences (1.5 kb) of *Ibbgal* genes was obtained from sweetpotato genomic DNA (https://ipomoea-genome.org/#), and then the cis-acting elements were predicted using the PLACE tool (http://www.dna.affrc.go.jp/PLACE/) [50].

Quantitative real-time PCR analysis

To validate the gene expression patterns observed in the transcriptomic experiments described above, we performed qRT-PCR analyses of these 17 *Ibbgals* genes. The primer sequences of the examined genes are listed in Table S2 (Additional file 2). Total RNA was extracted from the frozen samples by using an RNAprep pure plant kit (TIANGEN, Beijing, China) according to the manufacturer's instructions. qRT-PCR was performed using a Roche LightCycler® 480II system under the following conditions: 95°C for 15 s, followed by 40 cycles of 95°C for 15 s, 55°C for 15 s and 72°C for 15 s. The *Ib-Actin* gene was used as an internal reference to evaluate the relative gene expression level. The experiments were conducted for three replicates, and the data were calculated according to the $2^{-\triangle\triangle CT}$ method [51].

Plant materials and stress treatments

Tow cultivars of the sweetpotato (*Ipomoea batatas* (L.) Lam. *cv.* Jishu25 and Jishu29) plants were collected from the Crop Ressearch Institue, Shandong Academy of Agricultural Sciences, China. The uniform seedlings of these two cultivars were grown in the Hoagland solution at 26°C under a photoperiod of 16 h light/8 h dark. When the seedlings had five to six functional leaves and adventitious roots of 8 to 10 cm, these seedlings were subject to six different stresses, respectively. To study the expression patterns under these stresses, the adventitious roots of seedlings were submerged in the solution containing 150 mM NaCl, 20% PEG 6000, 100 mM ABA, 50 mg/L uniconazole, and 50 mg/L gibberellic acid (GA_3) respectively. For black spot pathogen treatment, *C. fimbriata* conidia was collected after growing in potato dextrose agar (PDA) at 28 °C for 7 days, then were diluted to 1×10^4 spores/mL with sterile water, and then the roots of sweetpotato seedlings were cultivated in the 1×10^4 spores/mL conidia suspension. The treated roots and leaves were collected after 0, 3, 6, 12, 24, and 48 h. In order to investigate the transcript levels of *Ibbgals* in different tissues, the fifth expanded leaves, lips, stems, fibrous roots and storage roots of these two cultivars were sampled at 125 days after transplanting. To analyze the expression profiles of *Ibbgals* in different stages of the storage root, the storage roots were sampled at 40, 55, 70, 95, 110, 125 and 150 days after transplanting in the sweetpotato field.

Statistical analysis

Statistical analysis was performed using the SPSS software package (v13.0), and the data were presented as means of three replicates. Differences between means were subjected to ANOVA, and the statistical significance of the difference between means was calculated with Duncan's new multiple ranges test and marked with asterisks at $p < 0.05$.

Abbreviations

ABA: abscisic acid; BLAST: Basic Local Alignment Search Tool; bgal: β-galactosidase; GH35: glycoside hydrolase 35; GA3: gibberellins; IAA: indolyl-3-acetic acid; JA: Jasmonic acid; MW: Molecular weights; NJ: Neighbor-Joining; pI: Isoelectric points; qRT-PCR: Quantitative reverse transcription polymerase chain reaction

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare they have no competing interests.

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Authors' contributions

FH designed and performed the experiments and wrote the paper. ZQ and TD performed some experiments and analyzed the data, AL and SD analyzed the data. TX, DM and QW revised the paper. ZL and LZ conceived the experiment.

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Tables

Table 1 Gene and protein analysis of bgals in *Ipomoea batatas.*

Gene name	CDS[a]	Length(aa)[b]	MW(kDa)[c]	pI[d]	Subcellular localization	Signal peptides[e]	N-glycosylation site[f]
Ibbgal1	2529	842	94.005	5.98	chloroplast	+	3
Ibbgal2	2196	731	81.393	8.39	chloroplast	+	2
Ibbgal3	2526	841	93.635	7.27	vacuole	+	1
Ibbgal 4	2529	842	93.578	8.71	vacuole	-	1
Ibbgal 5	2022	673	74.792	6.32	nucleus	-	1
Ibbgal 6	2529	842	93.665	7.94	chloroplast	+	1
Ibbgal7	2481	826	7.22	9.32	extracellular	+	4
Ibbgal8	2541	846	91.829	6.37	vacuole	+	2
Ibbgal9	2463	820	92.0858	5.31	vacuole	+	2
Ibbgal10	2391	796	89.004	6.83	nucleus	-	4
Ibbgal11	2505	834	94.335	8.57	chloroplast	+	5
Ibbgal12	2187	728	80.867	9.13	vacuole	+	2
Ibbgal13	3333	1110	125.149	5.5	chloroplast	-	6
Ibbgal14	2487	828	93.578	8.71	vacuole	+	5
Ibbgal15	2475	824	93.72	8.58	chloroplast	+	5
Ibbgal16	2412	803	89.731	6.34	chloroplast	+	6
Ibbgal17	2145	714	79.382	7.99	chloroplast	-	2

[a]The length of Ibbgals coding sequence

[b]The length of Ibbgals protein.

[c]Molecular weight

[d]Theoretical isoelectric point

[e]"+ " means contain signal peptide, "_" means lack signal peptide.

[f] Predicted using NetNGlyc.

Table 2. The putative cis-elements in the promoters of 17 *Ibbgal*s genes.

gene	Plant hormone response elements	Stress response elements	Light response elements	Other elements
lbbgal 1	ABRE[4], AuxRE[2], GARE[2], TATC-BOX, PYRIMIDINEBOXHVEPB1	box-W[2], MYC-like[18], ACGT[10]	INR[8], GT1-motif[5], Box 4 [8], IBOX[5], GBOX[3], GATAbox[10], GAG-motif, TCT-motif[3], Box II	EEs, TATA-box[21], GT[15], CCAAT-box[3], AAGAA-motif
lbbgal 2	GARE[4], TGACG-motif2, DPBFCOREDCDC3[2], CATATGGMSAUR[4]	MBS[2], MYC-like[18], ACGT[2]	INR [3], IBOX[2], GATAbox[14],GAG-motif,TBOX[2], TCT-motif[2],AT1-motif	Circadian[2], TATA-box[18], CCAAT-box[9], GCN4-motif, RY-element[4], GT[12]
lbbgal 3	ABRE,ERE, DPBFCOREDCDC3[3],	MYC-like[16], ACGT[2]	INR[2], GT1-motif, IBOX[6], DRE[2], GATAbox[15], GAG-motif, TBOX[3], TCT-motif, Box II[2]	Circadian, TATA-box[17], CCAAT-box[6], RY-element[2] , GT[12]
lbbgal 4	ABRE[5], GARE, AuxRE[2], PYRIMIDINEBOXHVEPB1	box-W, MYC-like1[8], ACGT[10]	INR8, GT1-motif5, Box 4[8], IBOX5, GATAbox10, GAG-motif, TCT-motif[3], Box II	EEs,TATA-box21,CCAAT-box3,GT[15], AAGAA-motif
lbbgal 5	ABRE[3], ERE, GARE, CGTCA-motif[2], TGACG-motif4, DPBFCOREDCDC3[4], PYRIMIDINEBOXHVEPB1	LRT, box-W, MYC-like1[2], ACGT[8], MBS[3] ,GT1[8]	INR6, GT1-motif2, Box 4[3], IBOX3, GATAbox15, Box A, TBOX,TCT-motif2, Box II2	Circadian3, TATA-box15, CCAAT-box6, Box A ,
lbbgal 6	ABRE[2], ERE, GARE[2], CGTCA-motif[2], TGACG-motif4, DRE2COREZMRAB17, PYRIMIDINEBOXHVEPB1	LRT[3], MYC-like[10], ACGT[12]	INR[4], GT1-motif, Box 4, IBOX[8], GATAbox[22], TBOX, TCT-motif[5], Box II[4]	TATA-box[21], CCAAT-box[4], RY-element, GT[13]
lbbgal 7	ERE, GARE[2], AuxRE, CGTCA-motif, TGACG-motif[3], DPBFCOREDCDC3[2], CATATGGMSAUR[2]	MYC-like[14], ACGT[4] , GT-1[5]	INR[4], Box 4[2], IBOX14, GATAbox[17]	Circadian[4], TATA-box[17], CCAAT-box[9], RY-element2
lbbgal 8	ABRE[3], ERE, GARE, DPBFCOREDCDC3[4], CATATGGMSAUR[4]	LRT[2], MYC-like[20], DRE[2], ACGT[12], MBS2 ,GT-1[9]	INR[3], GT1-motif, Box 4[4], IBOX[8], GATAbox[18], TCT-motif[3], Box II[3]	Circadian[2], TATA-box[20], CCAAT-box[3], RY-element

lbbgal 9	ABRE, ERE, GARE[2]	LRT[3], MYC-like[8], ACGT[6] , GT-1[5]	INR[3], GT1-motif, Box 4[2], IBOX[13],GATAbox[22], Tbox[2], Box II[3]	Circadian[5], EEs, TATA-box[28], CCAAT-box[3],GCN4-motif, RY-element[4]
lbbgal10	ABRE[2],GARE,DPBFCOREDCDC3, CATATGGMSAUR[2],PYRIMIDINEBOXHVEPB1	box-W, MYC-like[18], ACGT[12], MBS[3], GT-1[2]	INR[2], Box 4[3], IBOX[7]	TATA-box[16], CCAAT-box[3], RY-element[3], Box A[2]
lbbgal11	GARE[3],CATATGGMSAUR[2], PYRIMIDINEBOXHVEPB1	MYC-like[8], ACGT[4], MBS[2], GT-1[2]	INR[5], GT1-motif, Box 4[3], IBOX[7], GATAbox[18], GAG-motif, TBOX[2], TCT-motif, Box II	Circadian,TATA-box[23], CCAAT-box[4],AAGAA-motif, RY-element[2]
lbbgal12	ABRE[3], ERE, GARE[4], TGACG-motif, PYRIMIDINEBOXHVEPB1	LRT[3], box-W, MYC-like[18], DRE[4], ACGT[8],GT-1[8]	INR[8]GT1-motif, Box 43, IBOX3, GATAbox21, TCT-motif, Box II[2]	Circadian[2], TATA-box[27], CCAAT-box[3],RY-element
lbbgal13	ABRE[3], ERE, TGACG-motif, DPBFCOREDCDC3	LRT[2], MYC-like[18], ACGT[6], MBS[2], GT-1[4]	INR[4], GT1-motif[3], IBOX[15] , GATAbox[15], GAG-motif, TBOX, Box II[3]	Circadian, TATA-box[12], CCAAT-box[4], RY-element
lbbgal14	ABRE[3], ERE, GARE, TGACG-motif, DPBFCOREDCDC3[2], CATATGGMSAUR[4]	LRT[4], box-W, MYC-like[14], ACGT[6], MBS,GT-1[3]	INR[3], GT1-motif[2], Box 4, IBOX[10], GATAbox[18],CATT, TBOX[3], Box II[3]	Circadian, TATA-box[13], CCAAT-box[6], RY-element[3]
lbbgal15	GARE[2], DPBFCOREDCDC3[2]	LRT[3], box-W[2], MYC-like[28], GT-1[2]	INR[4], GT1-motif[2], IBOX[3], GATAbox[10], TBOX[2], TCT-motif, Box II	Circadian, TATA-box[2], CCAAT-box[5], RY-element
lbbgal16	ERE, GARE[2], DPBFCOREDCDC3[3], CATATGGMSAUR[2]	LRT[2], box-W, MYC-like[8], DRE[3], GT-1[6]	INR[4], Box 4[5], IBOX[2], GATAbox[13], GAG-motif, TBOX, TCT-motif	TATA-box[36], CCAAT-box[3], RY-element
lbbgal17	ABRE[7], ERE, GARE[3], TGACG-motif[4], DPBFCOREDCDC3[6], CATATGGMSAUR[2],	LRT[2], box-W[3], MYC-	INR[2], GT1-motif, Box 4, IBOX[9],	TATA-box[18], CCAAT-box[4],

GCCCORE		like[10], ACGT[6], MBS[2], GT-1	GATAbox[24], TBOX, Box II	GCN4-motif, RY-element[4]

Superscript numbers represent the repeats (2 or more than 2) of each cis-element in the *Ibbgal* promoter, while the others only contain one copy of corresponding cis-element.

ABRE and ACGT, cis-acting elements involved in the abscisic acid responsiveness;AuxRE, cis-acting regulatory element involved in auxin responsiveness; AAGAA-motif, cis-element involved in secondary xylem development; Box A, cis-acting elements of phenylalanine ammonia-lyase; Box II, part of a light responsive element; Box-W, fungal elicitor responsive element; Box 4, part of a conserved DNA module involved in light responsiveness; CATATGGMSAUR , cis-acting element involved in auxin responsiveness; CCAAT-box, MYBHv1 binding site; Circadian, cis-acting regulatory element involved in circadian control; DPBFCOREDCDC3, induced by ABA; DRE, cis-acting element involved in drought response;EEs, part of evening and circadian response; ERE, ethylene-responsive element; GARE, gibberellin-responsive element; GATA-motif,part of a light responsive element; Gbox, cis-acting regulatory element involved in light responsiveness; GATAbox, part of a light responsive element; GAG-motif, part of a light responsive element; GCCCORE , cis-acting element involved in jasmonate responsiveness; GCN4-motif, cis-regulatory element involved in endosperm; GT1-motif, light responsive element; GT-1, cis-acting element involved in the salt stress; INR, part of a light responsive element; IBOX, part of a light responsive element; LTR, cis-acting element involved in low-temperature responsiveness; MBS, MYB binding site involved in drought-inducibility; MYC-like , cis-acting elements of drought-responsive; PYRIMIDINEBOXHVEPB1, cis- and trans-acting elements involved in gibberellins and abscisic acid responsiveness; RY-element, cis-acting regulatory element involved in seedspecific regulation; TATA-box, core promoter element around −30 of transcription start; TATC-box, cis-acting element involved in gibberellin-responsiveness;TBOX, part of a light responsive element; TCT-motif, part of a light responsive element; TGACG-motif, cis-acting regulatory element involved in the MeJA-responsiveness.

Figures

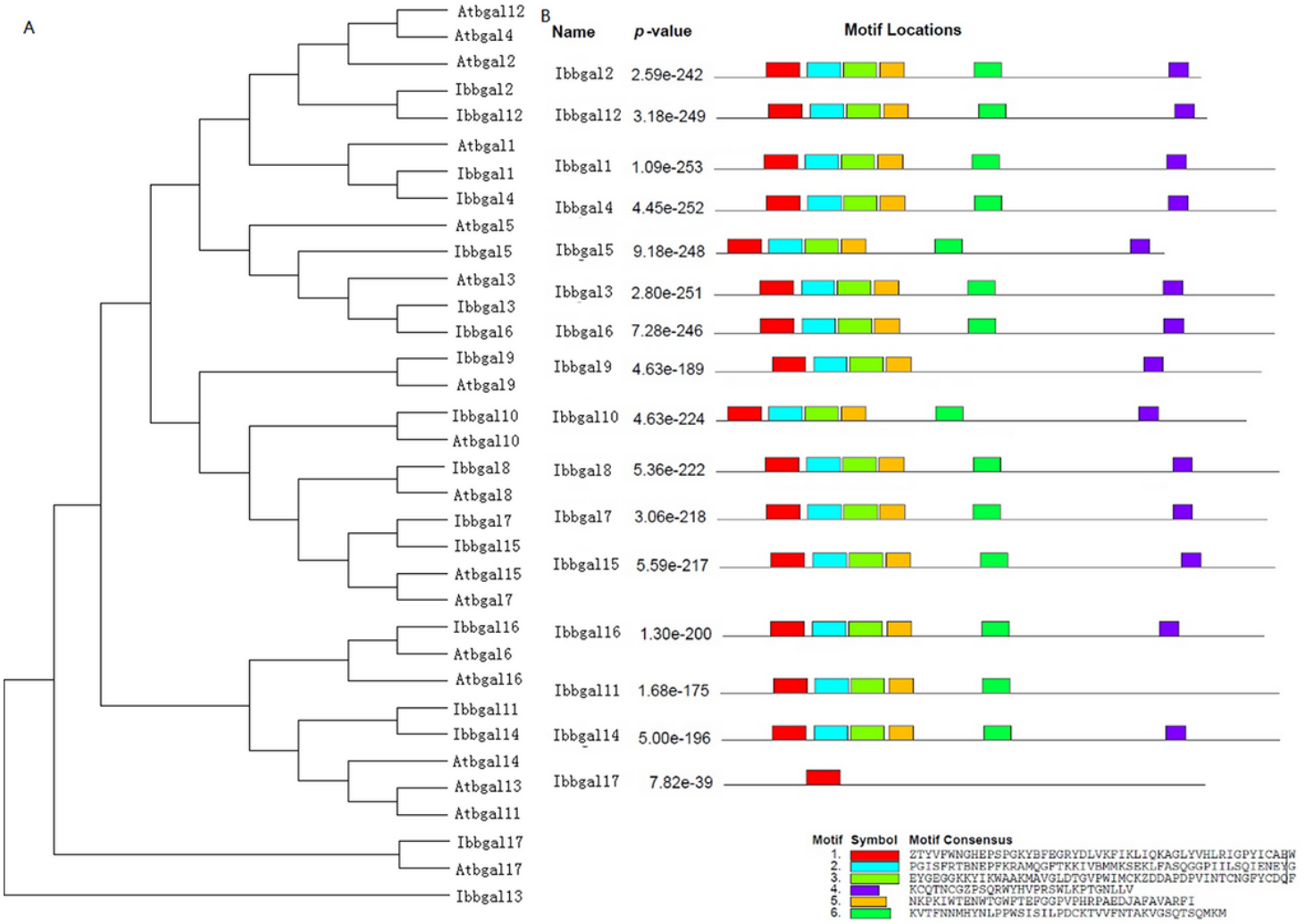

Figure 1

Phylogenetic relationship of Ibbgal proteins and motif distribution of Ibbgal genes. A: Phylogenetic relationship among sweet potato Ibbgal and A. thaliana Atbgals proteins. The unrooted tree was generated using MEGA7.0 by the NJ method, and the bootstrap test was set to 1000 replicates. B: Motif distribution in Ibbgal genes. The upper part represents the composition and position of motifs of Ibbgals with six motifs shown in distinct colors. The lower part shows the motifs of Ibbgals with the symbol of each residue.

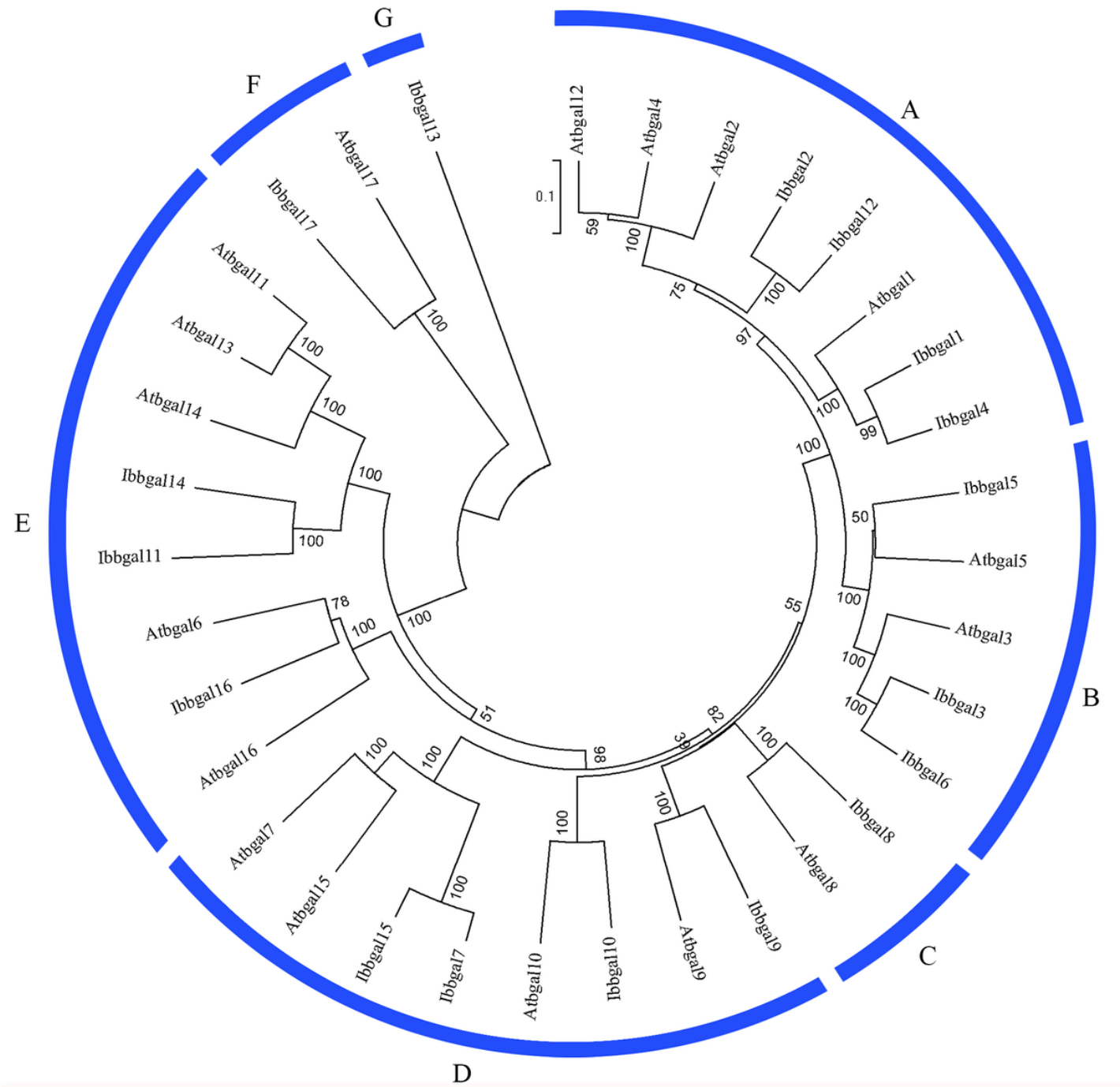

Figure 2

Phylogenetic tree of bgal proteins in sweetpotato, and Arabidopsis. The bgal protein sequences of Arabidopsis were downloaded from the database of Arabidopsis from the NCBI database (https://www.ncbi.nlm.nih.gov/).The tree was classified into 7 different subfamilies indicated by outer rings with blue color.

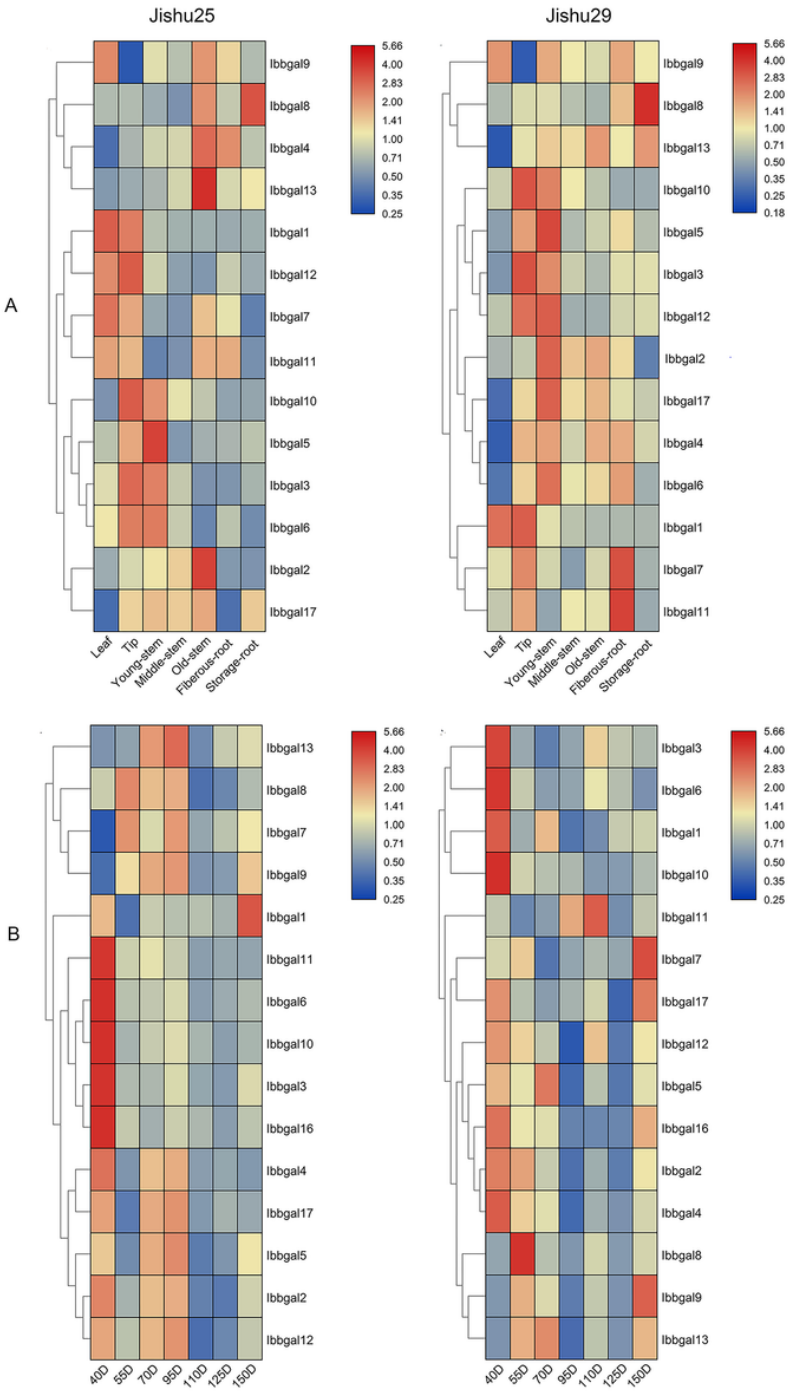

Figure 3

Relative mRNA expression levels of Ibbgal genes in tissues and storage root development of two sweetpotato varieties. A. Tissues including leaf, tip, young-stem, old-stem, fibrous root and storage root. B. Expression profiles in the storage root development .Gene expression were detected by quantitative real-time polymerase chain reaction. Log-transformed fold-change data were used for creating the heatmaps by TBtools. The coloured scale varying from blue to red indicates relatively low or high expression.

Figure 4

Expression patterns of Ibbgal genes under salt, drought stress and balck spot pathogen infection in the leave and root of the two cultivars. The y-axis represents relative expression. Bars represent the mean of three biological replicates ± SE.The asteridk indicated that the expression level between the treatment times is significantly different ($P<0.05$).

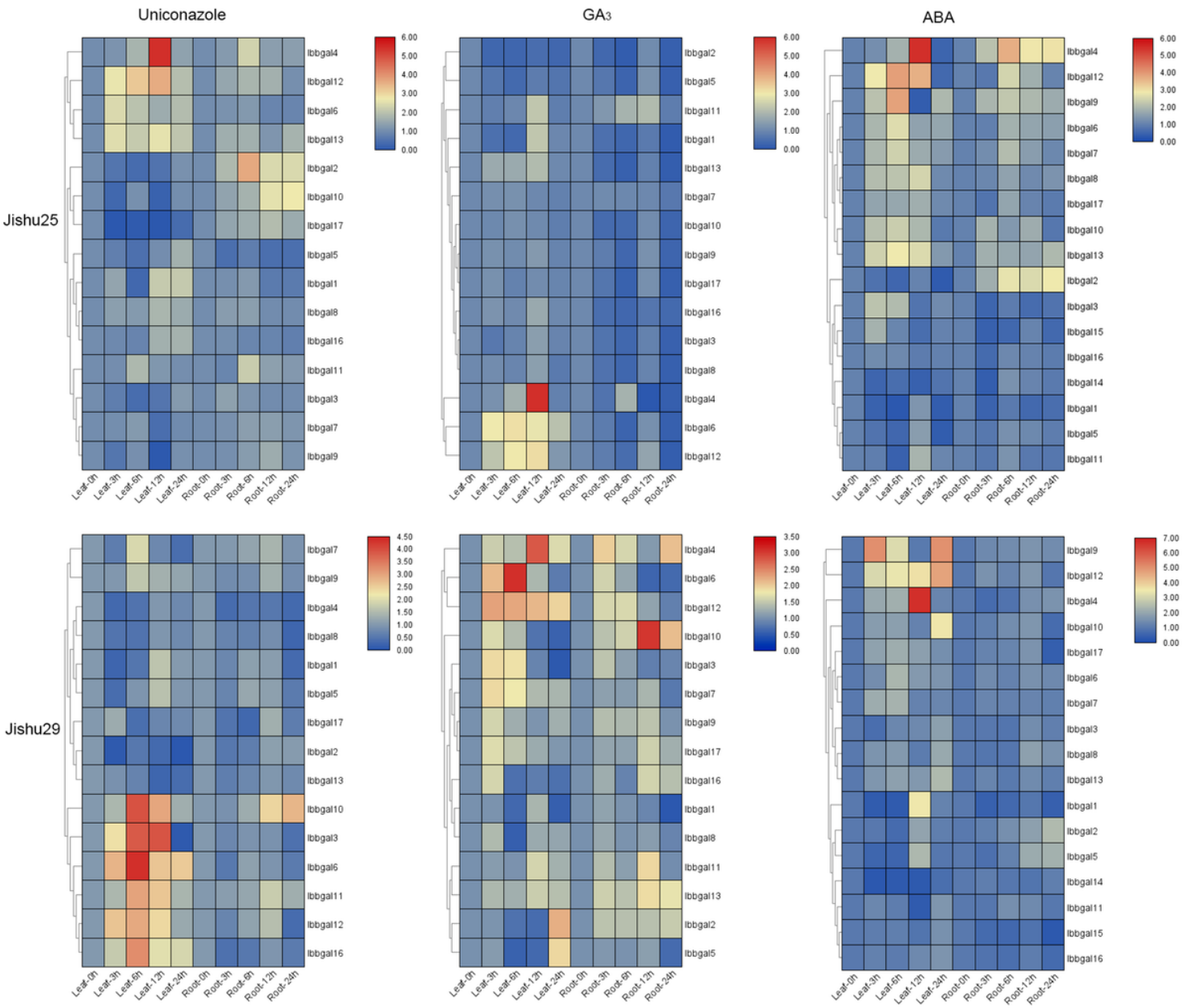

Figure 5

Expression profiles of lbbgal genes after uniconazole, GA3 and ABA treatment in the leave and root of the two cultivars. Gene expression was detected by quantitative real-time polymerase chain reaction. Log-transformed fold-change data were used for creating the heatmaps by TBtools. The coloured scale varies from blue to red, which indicates the low or high expression of each gene.

Supplementary Files

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