Genome-wide analysis of GATA factors in moso bamboo (*Phyllostachys edulis*) unveils that PeGATAs regulate shoot rapid-growth and rhizome development

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ABSTRACT

Background

Moso bamboo is well-known for its rapid-growth shoots and widespread rhizomes. However, the regulatory genes of these two processes are largely unexplored. GATA factors regulate many developmental processes, but its role in plant height control and rhizome development remains unclear.

Results

Here, we found that bamboo GATA factors (PeGATAs) are involved in the growth regulation of bamboo shoots and rhizomes. Bioinformatics and evolutionary analysis showed that there are 31 PeGATA factors in bamboo, which can be divided into three subfamilies. Light, hormone, and stress-related *cis*-elements were found in the promoter region of the *PeGATA* genes. Gene expression of 12 *PeGATA* genes was regulated by phytohormone-GA but there was no correlation between auxin and *PeGATA* gene expression. More than 27 *PeGATA* genes were differentially expressed in different tissues of rhizomes, and almost all *PeGATAs* have dynamic gene expression level during the rapid-growth

of bamboo shoots. These results indicate that *PeGATAs* regulate rhizome development and bamboo shoot growth partially via GA signaling pathway. In addition, *PeGATA26*, a rapid-growth negative regulatory candidate gene modulated by GA treatment, was overexpressed in Arabidopsis, and over-expression of *PeGATA26* significantly repressed Arabidopsis primary root length and plant height. The PeGATA26 overexpressing lines were also resistant to exogenous GA treatment, further emphasizing that PeGATA26 inhibits plant height from Arabidopsis to moso bamboo via GA signaling pathway.

Conclusions

Our results provide an insight into the function of GATA transcription factors in regulating shoot rapid-growth and rhizome development, and provide genetic resources for engineering plant height.

Running title: GATA family in moso bamboo

Keywords: GATA factors; gene family; rapid-growth; shoot; rhizome tissues; gibberellin; plant height; moso bamboo

Background

Moso bamboo is one of most-abundant non-timber forestry species and provides important resources for food, architecture, papermaking and fiber [1]. More importantly, moso bamboo is known for its explosive shoot growth rate, with a peak growth rate of 1 meter per day [1]. The rapid-growth shoot is largely dependent on the widespread rhizome system, which provide energy resources by absorbing from soil and more importantly, transporting from other rhizome-connected adult bamboos [2]. Therefore, studying the development of shoots and rhizomes will help us understand the rapid-growth regulation of bamboo and provide effective candidate genes for genetic manipulation of crop and forestry species.

The GATA factors play important roles in many developmental processes by binding to the consensus DNA sequence (A/T)GATA(A/G) to regulate gene expression at the transcriptional level [3, 4]. The GATA factors have a highly conserved type IV zinc finger DNA binding domain ($CX_2CX_{17-20}CX_2C$) and followed by a basic region [5-7]. In animals, GATA factors typically contain two zinc finger domains ($CX_2CX_{17-20}CX_2C$), while only the C-terminal domain has DNA binding function [5].

Animal GATAs are involved in development, differentiation, and control of cell proliferation [7]. However, the fungal GATA factors only contain a single zinc finger domain that is highly similar to the C-terminal zinc finger domain of the animal GATA factors [4, 8]. In plants, GATA factors contain $CX_2CX_{18}CX_2C$ or $CX_2CX_{20}CX_2C$ zinc finger domain [9, 10]. Interestingly, most of plant GATA factors have a single zinc finger domain, and very few of them also contain two zinc finger domains [9-11].

In animals, GATA factors involve in cell differential and organ development. Mutations in animal GATA factors cause severe developmental disorder diseases including anemia, deafness, renal and cardiac defects [12]. Fungal GATA factors play roles in nitrogen control, siderophore biosynthesis, light-regulated photomorphogenesis and circadian regulation [4].

Plant GATA factors originates from the identification of GATA motifs in regulatory regions of light and circadian clock responsive genes [13]. The first GATA factor identified in plant is NTL1 from *Nicotiana tabacum* [14]. GATA factors have been identified in many plant species, including Arabidopsis (29), rice (28), apple (35) and

soybean (64) [9, 10, 15]. Plant GATA factors are involved in many developmental processes, including plant architecture [16], flowering development [17], hypocotyl elongation [18] and seed germination [19]. Plant GATA factors employ several underneath molecular mechanisms, such as modulate nitrogen metabolism [14, 20], act as transcriptional regulator by either integrity of light and phytohormone signal transduction [21, 22] or direct involvement in phytohormone signal transduction to regulate plant growth [23].

Plant GATA factors regulate light signal transduction by combining with GATA promoter of light related genes [24, 25]. *GATA2* (At2g45050) has also been identified as a key transcriptional regulator of the integration of light and brassinosteroid signaling pathways [22]. Recent evidences suggest that GATA factors are involved in the regulation of plant hormone signal transduction. Two orthologous GATA-type transcription factors- GNC and CGA1/GNL from Arabidopsis thaliana were identified as GA-regulated genes [21, 23]. Loss-of-function mutants and overexpression lines of *GNC* and *GNL* are functionally related to germination, greening, and flowering time [17]. Chromatin immunoprecipitation (CHIP) results show that these two genes are

direct targets of PIF transcription factors, together with the fact that *gnc* and *gnl* loss-of-function mutations suppress *ga1* phenotypes, supporting that GNC and GNL are important repressors of GA signaling [21]. Another important phytohormone, auxin, is also regulated by GNC and GNL through functioning downstream of ARF2 [23]. In addition, the GATA factors are induced by cytokinin [26]. These results indicate that GATA factors play crucial roles in plant development and phytohormone-mediated growth. However, the role of GATA factors in rapid-growth and rhizome development remains elusive.

Recently, large-scale transcriptome analysis has shown that light and phytohormones may play important roles in the rapid-growth of bamboo [27-29]. In addition, a large number of transcription factor families are involved in the abiotic stress response and flower development have been studied in moso bamboo [30-32]. Although our group has functionally characterized rapid-growth associated key gene-*PeGSK1*, the rapid-growth associated transcription factor families are largely unexplored in moso bamboo.

In this study, we performed genome-wide survey of GATA factors in moso bamboo. A total of 31 GATA factors were identified in the moso bamboo genome. The phylogenetic relationship, gene structure and conserved domains of moso bamboo were systematically analyzed. The phytohormone-related *cis*-element and gene expression of *PeGATAs* under GA and auxin treatment were also characterized. More importantly, the gene expression of *PeGATAs* in different rhizome tissues and rapid-growth shoot were detailed analyzed. In addition, one of growth related PeGATA-PeGATA26 was overexpressed in Arabidopsis to functional validate its role in regulating plant height. Overall, our results provide information on the involvement of GATA factors in rhizome tissue development and rapid-growth shoot.

Results

Genome-wide characterization of GATA factors in moso bamboo

To identify the GATA factors in moso bamboo, the bamboo reference genome was used to scan the GATA factors using HMMER and blast tools (http://forestry.fafu.edu.cn/db/PhePacBio/download.php) [33]. A total of 31 potential GATA factors were identified in moso bamboo and named PeGATA1 to PeGATA31

Table 1 GATA factors in moso bamboo

Name	Gene ID	Location	ORF length(bp)	Size (aa)	MW (kDa)	PI	Sub-family
PeGATA1	PH01000001G0820	603239-605821(− stand)	762	253	27.2	7.11	□
PeGATA2	PH01000036G1110	651571-655167(+ stand)	1212	403	42.4	5.2	□
PeGATA3	PH01000040G1560	1013911-1015300(− stand)	801	266	28.7	9.77	•
PeGATA4	PH01000114G0660	460195-464981(+ stand)	912	303	32.2	5.96	•
PeGATA5	PH01000157G0800	521887-523791(− stand)	654	217	23.2	6.15	□
PeGATA6	PH01000162G1360	945504-954318(+ stand)	1500	499	56.4	9.4	□
PeGATA7	PH01000232G0180	85809-87165(− stand)	420	139	15.6	9.23	•
PeGATA8	PH01000242G0460	296415-297844(− stand)	648	215	22.6	8.2	•
PeGATA9	PH01000263G0760	473691-475362(− stand)	1095	364	37.5	7.72	□
PeGATA10	PH01000284G0590	365850-367843(− stand)	1131	376	39.3	5.89	□
PeGATA11	PH01000417G1130	669097-674119(− stand)	948	315	35.8	8.87	□
PeGATA12	PH01000468G1050	681872-683301(+ stand)	663	220	22.7	5.78	•
PeGATA13	PH01000604G0620	351426-352613(+ stand)	399	132	14.6	9.36	•
PeGATA14	PH01000750G0690	435897-442169(+ stand)	777	258	28.1	8.18	•
PeGATA15	PH01000836G0660	444348-452795(− stand)	1413	470	51.6	8.57	•
PeGATA16	PH01000985G0260	141878-143100(+ stand)	402	133	14.8	9.87	•
PeGATA17	PH01001002G0190	175975-177694(+ stand)	831	276	29.4	8.98	•
PeGATA18	PH01001129G0380	296297-297943(+ stand)	699	232	26	9.16	•
PeGATA19	PH01001155G0480	343290-344746(− stand)	741	246	25.3	9.66	□
PeGATA20	PH01001253G0390	263024-267886(− stand)	516	171	18.9	9.95	•
PeGATA21	PH01001451G0450	270274-272851(− stand)	930	309	32.6	8.68	□
PeGATA22	PH01001557G0370	244289-248001(+ stand)	594	197	20.7	9.14	•
PeGATA23	PH01001584G0350	297120-303918(− stand)	1035	344	37.8	4.75	•
PeGATA24	PH01001907G0160	109783-111580(+ stand)	1017	338	36.2	9.26	•
PeGATA25	PH01002105G0190	151795-153666(+ stand)	1248	415	44	8.6	□
PeGATA26	PH01002473G0050	19960-21655(− stand)	1011	336	36.1	9.64	•
PeGATA27	PH01002681G0110	59436-60698(− stand)	690	229	24	8.49	•
PeGATA28	PH01002830G0260	174984-177126(− stand)	381	126	13.8	9.69	•
PeGATA29	PH01003365G0100	53196-55342(+ stand)	369	122	13.3	9.4	•
PeGATA30	PH01003433G0110	88789-92096(− stand)	1167	388	39.8	9.33	□
PeGATA31	PH01004789G0060	58264-61570(− stand)	993	330	35	6.06	□

bp: base pair, aa: amino acids, MW: molecular weight, PI: isoelectric point, kDa: kilodalton

based on the chromosomal location. The CDS and protein sequences of PeGATA genes were listed in Additional file 1 and 2: Table S1 and S2. The detailed information of these PeGATA factors including length of CDS, size of amino acid, molecular weight (MW) of protein, gene location on chromosome and isoelectric point (PI) were listed in Table 1.

The length of CDS ranges from 366 bp to 1,500 bp, and the length of proteins ranges from 122 aa to 499 aa (Table 1). PeGATA29 is the smallest GATA protein with 122 amino acids, and the largest protein is PeGATA6 with 499 amino acids (Table S1). The predicted molecular weight of 31 PeGATA proteins ranges from 13.3 kDa (PeGATA29) to 56.4 kDa (PeGATA6) with an average size of 29.86 kDa (Table 1). The predicted PI of 31 PeGATA factors are all below 10.0, and the minimal protein is PeGATA23 with only 4.75 (Table 1).

To further investigate and characterize sequence conservation in the GATA proteins, multiple sequence alignments were performed using the amino acid sequences of the conserved GATA motifs in 31 PeGATAs (Fig. 1). Most bamboo GATA factors contain a single zinc finger domain. However, unlike Arabidopsis, several bamboo GATA factors contain multiple zinc finger domains (Fig. 2). Most of bamboo GATA factors contain 18 residues in the zinc finger loop ($CX_2CX_{18}CX_2C$), while five of them have 20 residues in the zinc finger loop ($CX_2CX_{20}CX_2C$) (Fig. 1). Interestingly, the gene subfamily analysis revealed that all of these five PeGATA factors all belong to the Class C type of the PeGATA family (Fig. 3). Similar to Arabidopsis and rice, moso bamboo

does not contain the animal- and fungal-type $CX_2CX_{17}CX_2C$ zinc finger domains (Fig. 1). Notably, five PeGATA genes factors have a defective GATA zinc finger domain (Fig. 1). PeGATA1 lacks the first Cys residue (-SHC) and PeGATA30 lacks the last Cys residue (CND-). Meanwhile, the GATA factors PeGATA14, PeGATA17 and PeGATA18 have only partial GATA motif (SRLTPAMRRGPTGPRSLCNAC for PeGATA14, CSDCNTTKTPLWRSGPCGPKAA for PeGATA17 and CSDCNTTKTPLWRSGP for PeGATA18) (Fig. 1). The observation is similar to the rice GATA factors as OsGATA24 also contains a partial GATA motif [9]. The results indicated that the bamboo GATA factors have a highly conserved GATA motif, especially compared to rice.

To further reveal the diversification of GATA genes in moso bamboo, putative conserved functional domain and motifs were also predicted in the NCBI conserved domain database and program MEME. Through MEME analysis, 10 motifs among the different gene subfamilies is shown in Fig. 2 and the identified multilevel consensus sequence for the motifs is shown in Additional file 3: Table S3. Motif 1 and 5 presented in 29 PeGATA proteins and they were annotated as conserved GATA zinc finger

domain $CX_2CX_{18}CX_2C$ and $CX_2CX_{20}CX_2C$, respectively (Fig. 2). Motif 5 was not found in PeGATA1, PeGATA14 and PeGATA23 by MEME (Fig. 2), which may be attributed to the zinc finger GATA subfamily domain corresponding to the conserved domain. Motif 2, 7 and 10 appeared nearly all members in subfamily I, and motif 4 and motif 6 only appeared in subfamily II (Fig. 2). Motif 3 was identified as the CCT domain and motif 9 was identified as TIFY domain (Fig. 2). These two domains were specific to subfamily III that was consistent with the classification by conserved domain as shown in Fig. 2. The identification of subfamily-specific motifs from bamboo GATA factors suggests that these motifs may contribute to the functional differences among different subfamilies.

Comparison analysis of the GATA subfamily among Arabidopsis, rice and moso bamboo

GATA factors in Arabidopsis and rice are classified in the clade A-D according to the residues of zinc fingers [9]. To determine the phylogenetic relationship among GATA genes in Arabidopsis, rice and moso bamboo, unrooted phylogenetic tree with 90 GATA factor sequences from all three species was constructed. The phylogenetic tree

182 analysis shows that all GATA factors have three major clades (Classes A, B and C) (Fig.
183 3). Among them, Class A is the largest clade and contains 38 members. In this clade,
184 twelve bamboo GATA factors (PeGATA1/2/5/6/9/10/11/19/21/25/30/31) clustered with
185 the Arabidopsis GATA factors AtGATA1, AtGATA2, and AtGATA4, which have been
186 reported to be involved in light regulation of gene expression and photomorphogenesis
187 [22, 34]. Class B formed the second largest clade containing 33 members and 13
188 bamboo GATA factors (PeGATA3/7/8/12/13/16/17/18/24/26/27/28/29) clustered with
189 the Arabidopsis GATA factors AtGATA21 (GNC) and AtGATA22. These two GATA
190 factors regulates phytohormone response, chlorophyll biosynthesis, starch production,
191 plant architecture, and nitrogen metabolism [17, 21, 23, 34, 35]. In Class C, six bamboo
192 GATA factors (PeGATA4/14/15/20/22/23) clustered with the Arabidopsis GATA factor
193 AtGATA25 (ZIM, Zinc-finger protein expressed in Inflorescence Meristem) and shows
194 the potential roles of hypocotyl and petiole elongation [18]. It is worth noting that no
195 bamboo GATA factor is found in Class D, which explains that bamboo GATA factors
196 may have different functions compared to Arabidopsis and rice.

197 **Gene structure of bamboo *GATA* genes in moso bamboo**

198 To determine the phylogenetic relationships among different members of the GATA
199 factors in moso bamboo, a phylogenetic analysis based on alignments of the 31
200 full-length GATA protein sequences was performed. As shown in Fig. 1 and Fig. 3, the
201 protein sequence alignment and neighbor-joining phylogenetic tree divides 31
202 PeGATAs into three clades according to the pattern of zinc finger domain or
203 homologous domains to the Arabidopsis and rice GATA factor families. The gene
204 structure of the *PeGATA* genes was shown in Fig. 4. The total exon numbers of
205 *PeGATAs* from each subfamily were calculated. Subfamily I comprised 12 members
206 with two or three exons except *PeGATA19* and *PeGATA6*. *PeGATA19* has only one
207 exon and *PeGATA6* has more then three exons with long introns. Subfamily II consists
208 of 13 members, and all of them contain two or three exons. Subfamily III was formed
209 by included 6 members with five to twelve exons (Fig. 4). The gene structure of GATA
210 factors is similar to that of rice [9]. Overall, the *PeGATA* genes contain exons ranging
211 from one to twelve in its CDS, and the gene structure is obviously different from each
212 other. The results indicated that the bamboo *GATA* genes have undergone significant
213 changes during its long evolutionary history.

Identification of hormone-related *cis*-elements in the promoter of the *PeGATA* genes

To further explore the function and regulatory pattern of the *PeGATA* gene, the PlantCARE database was used to scan the putative *cis*-elements inside the 1500 bp upstream of transcription start site. We categorized *cis*-elements into four categories based on their functions: light response elements, development, hormone and stress associated *cis*-elements (Fig. 5). The predicted *cis*-elements in *PeGATA* genes were closely related to the function of the GATA family in other plants [17, 19, 22, 23, 25]. Light responsive elements like G-box, GT1 and TCT were widely present in the promoter of *PeGATA* genes, and the G-box element has been reported to be involved in the regulation of chlorophyll II biosynthesis in Arabidopsis [36]. We also identified several hormone-responsive *cis*-elements such as ABRE [37], CGTCA-motif, TGACG, and TCA-elements (abscisic acid, MeJA and salicylic acid), and abiotic stress-responsive elements including ARE, GC-motif, LTR and MBS. In addition, tissue specific elements such as CAT-box, circadian responsive element and cell cycle regulation elements like MSA-like were also found in the promoter of the *PeGATA*

genes, which may have function in the regulation of plant morphology, flowering and growth [38]. Overall, *cis*-elements analysis indicated that bamboo GATA factors might be involved in response to light and phytohormone to regulate growth.

Transcription factors are typically located in the nucleus and regulate transcription of the target genes by binding to the *cis*-elements in their promoters. Consistent with our hypothesis, subcellular localization assays in tobacco showed that randomly selected bamboo *GATA* genes *PeGATA7, 20, 26* and *28* were clearly localized in the nucleus according to the GFP and DAPI stain signals (Fig. 6). Localization analysis revealed that bamboo GATA factors could also act as transcription factors to regulate gene expression.

Dynamic gene expression pattern of *PeGATAs* in rhizome tissues

The bamboo rhizome system can be divided into three groups: lateral buds, rhizome tips, and new shoot tips [2]. The widespread rhizome system is essential for rapid-growth of bamboo shoot through adopting and utilizing nutrients including nitrate [39]. The GATA factors are also related to nitrogen metabolism in other species [20], so we firstly checked if GATA genes expressing differentially in lateral buds,

246 rhizome tips, and new shoot tips. By analyze the RNA-seq data from our previous study
247 [2], we showed significant differential expression pattern of *PeGATA* genes among
248 different rhizome tissues (Fig. 7a, Additional file 4: Table S4). As shown in Fig. 7a, a
249 total of 15 *PeGATA* genes (*PeGATA1, 5, 9, 10, 14, 18, 19, 20, 21, 23, 25, 26, 27, 28* and
250 *29*) showed significantly higher expression in lateral buds than that from other two
251 tissues. Five *PeGATA* genes (*PeGATA6, 7, 8, 11* and *22*) highly expressed in the new
252 shoot tips, while reduced their expression in lateral buds. In the rhizome tips, seven
253 *PeGATA* genes (*PeGATA2, 3, 4, 15, 16, 24* and *30*) have remarkable higher expression
254 than other two tissues. In addition, *PeGATA12* and *31* expressed highly both in lateral
255 buds and rhizome tips, while slightly expressed in new shoot tips. Overall, 29 of the 31
256 *PeGATA* genes showed differential expression in three bamboo rhizome tissues,
257 suggesting that PeGATA factors may contribute to the growth regulation of rhizome.

258 **Expression profile of *PeGATAs* in bamboo under the treatment of exogenous**
259 **phytohormone**

260 GATA factors are closely related to phytohormones to regulate Arabidopsis growth and
261 development [21, 23], take together with the identification of phytohormone related

cis-elements in the promoter of the bamboo *GATA* genes (Fig. 5b), we rationally hypothesized that the *PeGATA* genes are also tightly regulated by phytohormones. To test our hypothesis, we performed gene expression analysis of the *PeGATA* genes under GA and auxin treatment based on the RNA-seq data published in the previous studies [28, 40]. A total of 12 *PeGATA* genes showed significant gene expression under GA treatment (Fig. 7b, Additional file 5: Table S5). Among them, the expression of *PeGATA7, 9, 10, 19, 30* and *31* was increased in GA_3 (100 μM) treated seedlings compared to that from untreated control (Fig. 7b). The largest difference was observed in *PeGATA10* (increased by 3.22-fold after GA_3 treatment). In contrast, six genes (*PeGATA1, 17, 18, 24, 25* and *26*) showed lower expression in GA_3-treated seedlings than control seedlings (Fig. 7b). *PeGATA26* was the most down-regulated gene with a 56% expression level reduction, and followed by *PeGATA18* with a 54% decline. It is worth noting that the other 19 *PeGATA* genes did not show significant expression change under GA treatment. These results indicate that the gene expression of *PeGATAs* is at least partially regulated by GA.

To test the relationship between *PeGATA* gene expression and auxin, we also analyzed the gene expression pattern of *PeGATA* genes under NAA treatment (5 μM NAA) in bamboo seedlings (Fig. 7c, Additional file 6: Table S6). Interestingly, unlike the results of GA treatment, only *PeGATA8* and *PeGATA9* showed significant gene expression change under auxin treatment. Although the expression levels of some other genes including *PeGATA1, 2, 10, 16* and *22* were slightly changed, the gene expression of most *PeGATA* genes did not change under auxin treatment (Fig. 7c), suggesting that PeGATAs may not be affected by auxin. Overall, these results suggest that *PeGATAs* are partially regulated by GA, but are not affected by auxin.

Genes expression pattern of *PeGATAs* in the rapid-growth of bamboo shoots

As the rapid-growth of bamboo shoots is largely determined by phytohormone and nutrients [2, 27], and we have demonstrated that *PeGATAs* are differentially expressed in rhizome tissues and under GA treatment (Fig. 7a, b), we hypothesized that *PeGATAs* may also be involved in fast-growing bamboo shoots. To validate our hypothesis, the expression profiles of the *PeGATAs* in the fast-growing bamboo shoots (0.15 m, 0.5 m, 1.6 m, 4.2 m and 9 m) were examined by qRT-PCR (Fig. 8). The results showed that

293 almost all GATA genes changed their gene expression in at least one of fast-growing
294 stages. Among them, seven *PeGATA* genes (*PeGATA1, 3, 9, 12, 14, 16, 26* and *31*)
295 continued to decrease their gene expression with the increase of shoot height (Fig. 8).
296 The best example is *PeGATA9*, which showed over 30-fold expression reduction in
297 9-meter shoots compared to 0.15-meter shoots. The results indicate that these *PeGATAs*
298 genes may be negatively correlated with shoot height. Another groups of *PeGATA*
299 genes (*PeGATA5, 6, 7, 8, 11, 15, 16, 20, 21, 22, 23, 24, 27, 28, 29* and *30*) showed
300 minimal gene expression at the middle growth stages. The results indicate that these
301 *PeGATAs* play an important role in the negative regulation of shoot growth at the
302 middle shoot development stages. Another sets of *PeGATA* genes (*PeGATA2, 4, 10* and
303 *19*) increased their expression at early shoot developmental stages, and then reduce
304 their expression along with the increase of shoot heights. Finally, three *PeGATA* genes
305 (*PeGATA17, 18* and *27*) were increased their expression during early shoot
306 developmental stages, then reduced their expression during middle developmental
307 stages, and then increased their expression again during late shoot developmental
308 stages. Interestingly, we did not find that any *PeGATA* genes continued to increase its

309 expression along with bamboo shoot development. Overall, our results indicate that
310 *PeGATAs* genes may be negatively correlated with rapid-growth of bamboo shoots.

311 **Over-expression of *PeGATA26* negatively regulates plant height in Arabidopsis**

312 To understand the function of PeGATA factors, we chose PeGATA26 as an example to
313 verify the role of PeGATA factors in plant growth. *PeGATA26* showed higher gene
314 expression in the growth-inactive lateral buds than the growth-active rhizome tips and
315 new shoot tips (Fig. 7a). Moreover, *PeGATA26* have lower gene expression under GA
316 treatment (Fig. 7b), and its expression decreased along with rapid-growth of bamboo
317 shoots (Fig. 8). These results suggest that *PeGATA26* act as a negative regulator of plant
318 growth and height in moso bamboo. Therefore, we hypothesized that *PeGATA26* plays
319 a crucial role in regulating plant growth. In a previous study, we successfully
320 characterized one of the fast growth-suppressing genes *PeGSK1* by over-expressing it
321 into Arabidopsis [1]. Therefore, we used a similar strategy to verify the function of
322 *PeGATA26* in regulating plant growth by over-expressing it into Arabidopsis.

323 The homozygous T3 transgenic lines were used to analyze phenotype, and
324 over-expression of *PeGATA26* resulted in a significant growth retardation phenotype

(Fig. 9a). Gene expression of *PeGATA26* was successfully detected by qRT-PCR (Fig. 9b). The phenotypes between the two over-expressing lines were similar and the intensity of phenotype correlated with the expression of each transgenic lines (Fig. 9a). Therefore, *PeGATA26* over-expressing line 1 (PeGATA26-ox1) with a stronger phenotype was used for further detailed phenotypic analysis. Interestingly, PeGATA26-ox1 showed a significant dwarf phenotype with a dramatic shorter inflorescence compared to the control (Fig. 9c), indicating that *PeGATA26* inhibits growth of plant height. Moreover, *PeGATA26* also repressed primary root growth (Fig. 9c). However, *PeGATA26* promoted Arabidopsis hypocotyl length (Fig. 9c). These results indicate that *PeGATA26* regulates plant growth in a tissue-specific manner: repressing cell growth in roots and inflorescences, while promoting cell growth in hypocotyls.

As *PeGATA26* was down-regulated under the GA treatment in bamboo seedlings (Fig. 7b), we subsequently analyzed whether PeGATA26 was also regulated by GA in Arabidopsis. Interestingly, exogenous GA treatment did not recovery the dwarf phenotype of PeGATA26-ox1 (Fig. 9d), indicating that PeGATA26 is negatively

correlated with GA to regulate plant height in Arabidopsis. Similar to the results of its Arabidopsis orthologous gene [21], the gene expression pattern of the GA signaling pathway genes in PeGATA26-ox1 were also similar to those observed in the GA biosynthesis mutants- *ga1* (Fig. 9e). The results support that PeGATA26 also repressed GA signaling downstream of the DELLA protein. These results suggest that PeGATA26 inhibits plant root and stem growth in Arabidopsis, take together with its gene expression negatively correlated with the growth of rhizome tissues and shoot in moso bamboo, we concluded that PeGATA26 is a negative growth regulator for plant height control from Arabidopsis to moso bamboo.

Discussion

Moso bamboo is one of important non-timber forestry species with great value in providing food and building materials [33]. Moreover, bamboo is known for its fast-growing shoots and widespread rhizomes [2]. It has been reported that several gene families are involved in flower development and abiotic stress [30-32, 41], the rapid-growth associated transcription factors remain elusive. The genome sequences of

357 moso bamboo[33] and transcriptome studies [2, 27, 28, 40] provide important
358 platforms for the identification of rapid-growth shoot and rhizome development
359 associated gene families. The rapid-growth related genes could provide useful
360 information for genetic manipulation of plant height in future.

361 GATA factors have key functions in developmental control and response to the
362 environmental stresses [16, 17, 19]. In this study, we characterized 31 GATA factors
363 from the moso bamboo (Table 1), and PeGATA factors have highly conserved zinc
364 finger protein domains compared to Arabidopsis and rice GATA factors (Figs. 1-4).
365 More importantly, gene expression analysis of *PeGATAs* in different rhizome tissues
366 and fast-growing bamboo shoots showed that several *PeGATAs* had negative
367 expression patterns with bamboo shoots and rhizome growth (Figs. 7a, 8). Furthermore,
368 the gene expression of *PeGATAs* was partially dependent on phytohormone-GA in
369 bamboo (Figs. 7b). Moreover, overexpression of *PeGATA26* in Arabidopsis repressed
370 the growth of root and plant height in a GA dependent manner (Fig. 9). Overall, our
371 results indicate that *PeGATAs* are involved in regulating the growth of plant height
372 from Arabidopsis to moso bamoo probably through GA signaling pathway.

373 Bioinformatics analysis showed that there were 31 PeGATA factors in moso bamboo
374 (Fig. 1). The number of bamboo GATA factors was closer to other species, including
375 Arabidopsis (29), rice (28) and apple (35) [9, 10, 15]. Furthermore, most of PeGATA
376 factors have a conserved single $CX_2CX_{18-20}CX_2C$ zinc finger domain that is highly
377 similar to that from Arabidopsis and rice (Fig. 1). In addition, the subfamily of I to III
378 from moso bamboo showed a highly evolutionary conservation compared to
379 Arabidopsis and rice (Fig. 3). These results indicate that most of the GATA factors from
380 moso bamboo are conserved compared to other species. However, unlike containing
381 only one zinc-finger domain in subfamily I GATA factors from Arabidopsis and rice [9],
382 PeGATA6 and PeGATA11 from the bamboo GATA subfamily I have two GATA-type
383 zinc finger domains (Fig. 1). Moreover, more protein domains from the bamboo GATA
384 subfamily III were identified compared to that from Arabidopsis and rice (Fig. 2).
385 Interestingly, a unique feature of the PeGATA factors is that they only have three
386 subfamilies compared to the four subfamilies of Arabidopsis and rice (Fig. 3). These
387 differences suggest that PeGATAs do have certain specificity compared to that from
388 Arabidopsis and rice. Future analysis the functions of GATA factors, including

389 AtGATA26, AtGATA27 and OsGATA30 from subfamily IV (Fig. 3), can help us reveal
390 why bamboo lacks these GATA factors.

391 The first GATA factor is identified according to the light and circadian clock related
392 *cis*-elements in its promoters [13]. Thus, the function of the GATA factors can be
393 predicted based on the identification of *cis*-elements from their promoter. In this study,
394 we found that the promoter of *PeGATAs* has many important *cis*-elements, including
395 light responsive element, cell cycle regulation and phytohormone responsive elements
396 (Fig. 5), which are closely related to the regulation of plant growth. Thus, *PeGATAs*
397 may be involved in regulating plant growth through these *cis*-elements to affect their
398 gene expression and further downstream genes.

399 The bamboo has a well-established rhizome system to develop new shoot tips and
400 widespread rhizome tips [2, 39]. However, the lateral buds of bamboo rhizomes are not
401 active and dominant in growth [2]. Therefore, identification of GATAs with different
402 expression patterns in these tissues will help us clarify the role of GATA factors in
403 rhizome development, which remains unclear. In this study, we found that 15 *PeGATA*
404 genes are highly expressed in lateral buds (Fig. 7a). Among them, *AtGATA2*

(orthologous gene of *PeGATA9*) has been reported to have a function to restrict cell division in the proliferation domain of Arabidopsis root meristem [42], and high expression of *PeGATA9* in lateral buds indicates that *PeGATAs* may also be involved in inhibiting the cell division in bamboo lateral buds (Fig. 7A). In contrast, the lower expression of *PeGATA9* in the actively growing new shoot tips and rhizome tips (Fig. 7A), suggesting a negative correlation between *PeGATA9* and cell growth in bamboo rhizome. It has been reported that *AtGATA22*, a orthologous gene of *PeGATA18*, is involved in response to cytokinin and negatively regulates root growth in Arabidopsis [43], we found that *PeGATA18* has higher gene expression in lateral buds (Fig. 7a), suggesting that *PeGATA18* may play a role in negative regulation of lateral buds cell growth. Similarly, the orthologous gene of *PeGATA26* also plays a negative role in elongation growth [21]. Overall, these results suggest that these 15 *PeGATAs* may contribute to negatively regulating the growth of lateral buds. Next, five *PeGATAs* were highly expressed in the new shoot tips than the other two tissues (Fig. 7a). Among them, the mutation of *GATA19* (orthologous gene of *PeGATA8*) in Arabidopsis causes meristem defects [44]. Here, the high expression of *PeGATA8* in new shoot tips (Fig.

7a), suggests that *PeGATA8* may also be involved in the regulation of shoot meristem development in bamboo. Furthermore, seven *PeGATAs* were highly expressed in the rhizome tips, indicating that they are involved in the growth of the rhizome tips (Fig. 7a). Overall, more than 87% of *PeGATAs* (27/31) was highly expressed in one of rhizome tissues (Fig. 7a). The results indicate that *PeGATAs* strongly participate in the regulation of rhizome growth. Once the transformation system is ready in future, functional characterization of these *PeGATAs* in moso bamboo will help us elucidate the exact role of PeGATAs in rhizome growth control.

The correlation between GATA factors and GA or auxin has been extensively studied in Arabidopsis [21, 23, 42, 45]. In this study, we found that gene expression of 12 *PeGATAs* changed under GA treatment, while only two *PeGATAs* responded to auxin treatment (Fig. 7b, c). In addition, motif analysis indicated that the promoter of *PeGATAs* has more GA-related *cis*-elements than auxin (Fig. 5b). Our results indicate that GA rather than auxin frequently regulates the expression of *PeGATAs* in moso bamboo.

436 Gene expression analysis showed that almost all PeGATAs have changed their
437 expression during the rapid-growth of bamboo shoots (Fig. 8). For example, *GATA2*
438 (orthologous gene of *PeGATA9*) limits cell division in root meristem of Arabidopsis
439 [42], and the expression of *PeGATA9* was down-regulated over 30 times in late
440 rapid-growth stage (9 m) than the early stage (0.15 m) (Fig. 8). The results indicate that
441 *PeGATA9* may also negatively regulate the rapid-growth of bamboo shoot.
442 Identification of many rapid-growth related *PeGATAs* indicates that *PeGATAs* are
443 involved in regulating the bamboo shoot. The rapid-growth of bamboo shoot is tightly
444 controlled by phytohormone [27]. Current studies reveals that ABA is the only negative
445 regulator of fast-growing shoots, while BR, auxin, GA and cytokinin antagonize with
446 ABA to promote rapid-growth of bamboo shoots [27]. Interestingly, all of the 12
447 GA-related *PeGATAs* showed differential expression in at least one of rapid-growth
448 stages (Figs. 7b, 8), suggesting that GA may regulate rapid-growth of bamboo shoots
449 via modulating gene expression of *PeGATAs*. To understand the function of GA-related
450 *PeGATAs* in plant height control, *PeGATA26* was selected to validate its role in
451 Arabidopsis growth (Fig. 8). Overexpression of *PeGATA26* in Arabidopsis resulted in

growth retardation phenotypes such as dwarfism and shorter primary root length, and the *PeGATAs* over-expressed lines was resistant to GA treatment (Fig. 8). Overall, these results further support that *PeGATAs* could regulate plant heights from Arabidopsis to moso bamboo via GA signaling pathway.

Conclusions

With the explosive growth rates of bamboo shoots and widespread rhizomes, the identification of key regulatory genes in the bamboo shoot and rhizome growth control will provide important genetic resources for the genetic manipulation of plant height. In this study, we characterized 31 GATA factors from moso bamboo. More importantly, the gene expression of *PeGATAs* is closely related to the development of rhizome tissues and rapid-growth of bamboo shoots. Moreover, the gene expression of *PeGATAs* was partially regulated by the phytohormone-GA in bamboo. In addition, functional characterization of *PeGATA26* in Arabidopsis provides insight into how *PeGATAs* regulate plant height from Arabidopsis to bamboo via the GA signaling pathway. However, we also noticed that GA regulates expression of only part of PeGATAs. As ABA-related *cis*-elements are more widespread than GA, and ABA is the

only known negative regulatory hormones in the rapid-growth control of bamboo shoots, we cannot rule out that *PeGATAs* may also regulate plant height through ABA signaling pathway. In summary, our results provide certain evidence that GATA transcription factor regulate the development of rhizome tissues and the rapid-growth of bamboo shoots.

Methods

Identification of GATA factors in moso bamboo

To identify the GATA factors, the genome and protein sequences of moso bamboo were downloaded from BambooGDB database (http://forestry.fafu.edu.cn/db/PhePacBio/download.php) [33]. GATA protein sequences from Arabidopsis and rice were obtained from previous published data [9]. We performed multiple sequence blast and alignment with an expected value of 10. The HMMER profile of the GATA domain (PF00320) from Pfam (http://pfam.xfam.org/) was used to search the bamboo protein database with a threshold: e-values < 10^{-5} [46]. The bamboo GATA factors were determined with the criteria: it is present in both blast and HMMER motif analysis lists. The number of amino acid, molecular weights (MWs)

and isoelectric points (PI) of bamboo GATA factors were predicted by ProtParam (https://web.expasy.org/protparam/).

Phylogenetic tree, conserved domain, motif recognition and *cis*-elements analysis

Multi-sequence alignment of the GATA protein sequences was carried out by ClustalX [47], and phylogenetic tree was constructed using MEGA7 by the Neighbour-Joining method (bootstrap analysis for 1000 replicates) [48]. Conserved domains were obtained from NCBI (https://www.ncbi.nlm.nih.gov/cdd) [49] and motifs were analyzed using MEME with default parameters (version 5.0.5, http://meme-suite.org/tools/meme) [50]. For *cis*-elements analysis, DNA sequences from 1.5-kb upstream region of each *PeGATA* gene were used to scan any potential *cis*-element using the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) [51].

Subcellular localization analysis

To verify the location of PeGATAs, the full-length CDSs without stop codon from four *PeGATA* genes were cloned into a modified pCambia3301 vector with C-terminal GFP as described in our previous study [1]. The *ACTIN2::PeGATAs*-GFP and the *ACTIN2*::GFP control constructs were then transiently transformed into tobaccos, and

GFP and DAPI fluorescence was observed using a microscope (20x, Zeiss, LSM880). .

Gene expression analysis

To investigate gene expression levels of the PeGATA genes in different tissues or hormone treatments, RNA-seq data was downloaded from Short Read Archive (SRA) database for the lateral buds, rhizome tips and new shoot tips (SRP093919) [2], and bamboo seedlings under GA and auxin treatment (SRP119416 and SRP109631) [28, 40], respectively. The pair-end reads were mapped to the moso bamboo reference genome using tophat2, and differential expressed genes were detected by cufflinks with defaults parameters [52].

Plant materials and qRT-PCR analysis

The moso bamboo shoots used in this study were collected in JianOu County (E118º28'; N27º00'), Fujian Province, China. The middle internode of different height of bamboo shoots were sampled and stored in liquid nitrogen immediately.

qRT-PCR analysis was performed for each member of the GATA family genes during the rapid-growth of bamboo shoots. Total RNA was extracted from the bamboo

samples using HiPure Plant RNA Mini Kit (Magen, R4151-02) and 1μg RNA was taken for reverse transcription into cDNA using a commercial Kit (Monad, RN05004M). Primers for qRT-PCR were designed on Primer3 (http://primer3.ut.ee/) using the CDS of each PeGATA gene. qRT-PCR were performed using MonAmp™ ChemoHS qPCR Mix (Monad, RN04002M) in a 20 μl reaction. The following program was used for qRT-PCR: 95 °C for 5 min; 40 cycles of 95 °C for 10 s, 60 °C for 10 s and 72 °C for 30 s.

Ectopic expression analysis

The *PeGATA26* was cloned and expressed in Arabidopsis exactly following the procedures for the *PeGSK1* in our previous study[1]. The T3 generation seedlings were used for phenotype analysis. The primary root length, plant height and hypocotyl length were measured using ImageJ[1].

The primers used in this study were listed in Additional file 7: Table S7.

Declarations

Competing interests

The authors declare that they have no competing interests.

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Author contributions

T.W., C.L., and L.M. conceived the ideas. T.W. and W.W. performed the experiments. T.W., Y.Y., S.L. and Z.Z. contributed to data analysis. T.W. and L.M. wrote the manuscript.

Figure legends

Fig. 1 Alignment of the amino acid sequences of bamboo GATA factors. The GATA motifs and amino acid positions are marked with a box and an asterisk. The sequence identities of GATA motifs are shown at the bottom.

Fig. 2 Schematic diagram of conserved domain analysis in bamboo GATA proteins.

548 Each color represents a different motif.

549 **Fig. 3** Phylogenetic analysis of GATA factors in bamboo, rice and Arabidopsis. The
550 phylogenetic tree was made based on the amino acid sequences using MEGA7.0 by
551 the neighbor-joining method with 1000 bootstrap replicates. The tree shows four
552 major phylogenetic classes (Classes A to D) indicated by different colors.

553 **Fig. 4** Phylogenetic analysis and gene structure of bamboo GATA factors. (a)
554 Phylogenetic tree construction of the PeGATA factors based on the amino acid
555 sequences using MEGA 7.0. The tree showed three major phylogenetic subfamilies
556 (subfamilies I to •) , represented by different colored backgrounds. (b) CDS/UTR
557 structure of the *PeGATA* genes. The yellow and green boxes indicate exons and UTRs,
558 and the black lines represent introns. The size of exons and introns can be estimated
559 using the scale at the bottom.

560 **Fig. 5** *Cis*-elements analysis in the promoter of bamboo *GATA* genes. (a): Overview
561 of the main types of cis-elements identified from the 1.5-kb upstream sequence of the
562 bamboo *GATA* genes by the PLANTCARE database. (b): Hormone related
563 *cis*-elements were analyzed and each colored block with numbers represents the

number of *cis*-elements in the bamboo GATA promoter.

Fig. 6 Subcellular localization analysis of bamboo GATA factors. The bamboo *GATA* genes were cloned and constructed in a modified pCambia3301 vector with a C-terminal GFP fusion. These vectors were transformed into tobacco, and the GFP and DAPI signals were captured from the identical areas by microscopy (20x).

Fig. 7 Expression profiles of bamboo *GATA* genes in different tissues and hormone treatment. (a): The gene expression of 31 bamboo *GATA* genes in different rhizome tissues was presented by heatmap. (b) and (c): The expression of bamboo GATA genes in the seedlings under GA and auxin treatment. Expression values were normalized and presented at the right side, and green represents lower expression level and red indicates a higher expression level.

Fig. 8 The expression level of *GATA* genes in bamboo shoots. The gene expression values were detected by qRT-PCR. The Y-axis and X-axis indicate relative expression level at different height of shoots. S1: 0.15 m shoots; S2: 0.5 m shoots; S3: 1.6 m shoots; S4: 4.2 m shoots; S5: 9 m shoots.

Fig. 9 Ectopic expression of *PeGATA26* inhibits the plant height of Arabidopsis. (a):

580 Overexpression of *PeGATA26* resulted in a dwarf phenotype in Arabidopsis. (b):
581 The expression level of *PeGATA26* were detected in both transgenic with the *ACTIN2*
582 gene as an internal control. (c): Phenotypic analysis of plant height, hypocotyl length
583 and primary root length in PeGATA26 overexpressing line 1 compared to control.
584 They all have significant differences compared to the control by the t-test: $P \leq 0.0001$,
585 which was represented by four stars in the figure. (d): Overexpression of *PeGATA26*
586 repressed plant height of Arabidopsis and PeGATA26-ox1 was resistant to exogenous
587 GA treatment. (e): The expression of GA signaling genes in the PeGATA26-ox1 was
588 similar to that of GA biosynthesis mutants-*ga1*. The expression of GA signaling genes
589 was detected by qRT-PCR and the *ACTIN2* was used as the internal control.
590

591 **SUPPORTING INFORMATION**

592 **Additional files**

593 **Additional file 1: Table S1.** The coding region sequences of *PeGATA* genes.

594 **Additional file 2: Table S2.** The amino acid sequences of PeGATA factors.

595 **Additional file 3: Table S3.** List of protein motifs identified in PeGATA factors.

596 **Additional file 4: Table S4.** Gene expression of *PeGATA* genes in different rhizome
597 tissues.

598 **Additional file 5: Table S5.** Gene expression of *PeGATA* genes under GA treatment.

599 **Additional file 6: Table S6.** Gene expression of *PeGATA* genes under auxin
600 treatment.

601 **Additional file 7: Table S7.** List of primers used in this study.

602

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Fig.1

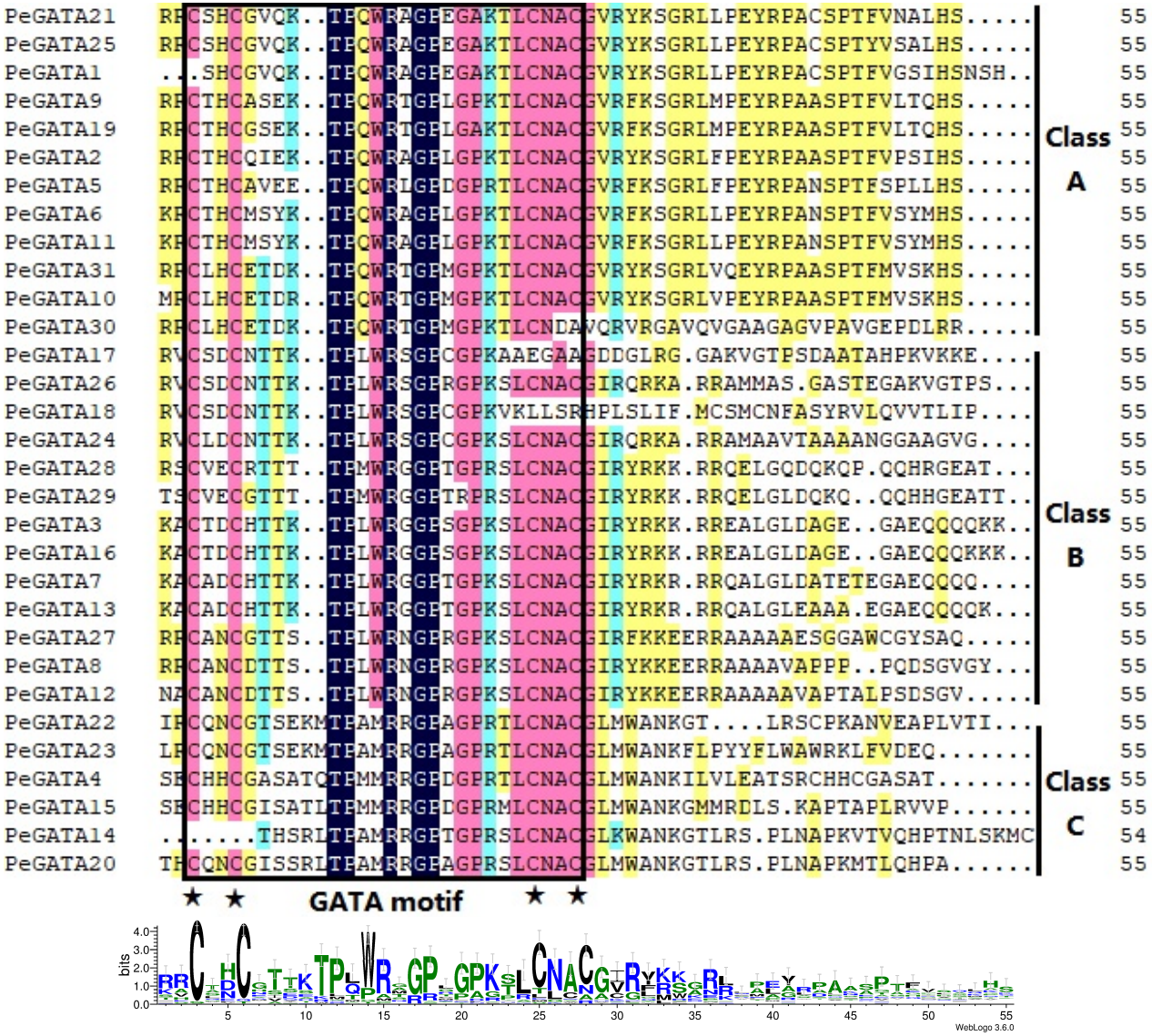
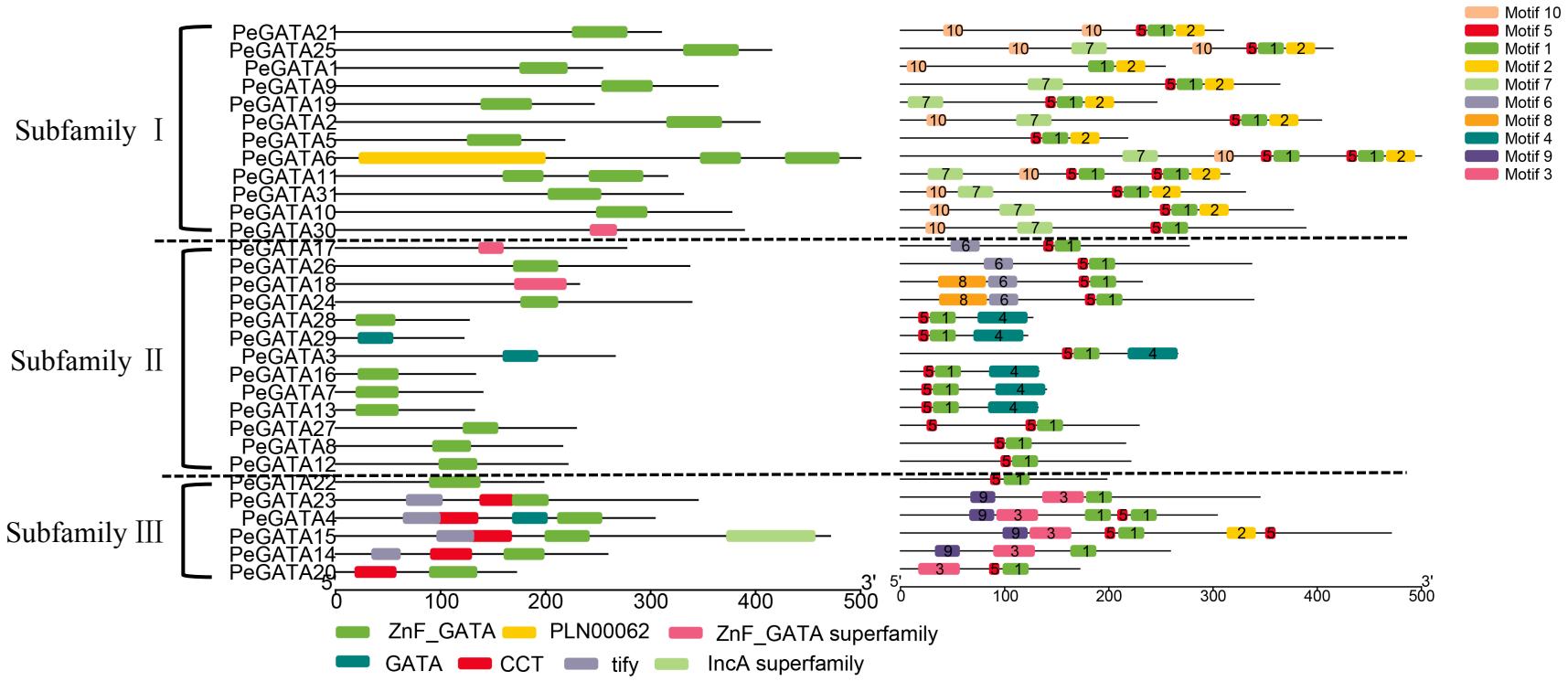

PeGATA21 RRCSHCGVQK..TECWRAGPEGAKTLCNACGVRYKSGRLLPEYRPACSPTFVNALHS..... 55
PeGATA25 RRCSHCGVQK..TECWRAGPEGAKTLCNACGVRYKSGRLLPEYRPACSPTYVSALHS..... 55
PeGATA1 ...SHCGVQK..TECWRAGPEGAKTLCNACGVRYKSGRLLPEYRPACSPTFVGSIHSNSH.. 55
PeGATA9 RRCTHCASEK..TECWRTGPLGPKTLCNACGVRFKSGRLMPEYRPAASPTFVLTQHS..... 55
PeGATA19 RRCTHCGSEK..TECWRTGPLGAKTLCNACGVRFKSGRLMPEYRPAASPTFVLTQHS..... 55
PeGATA2 RRCTHCQIEK..TECWRAGPLGPKTLCNACGVRYKSGRLFPEYRPAASPTFVPSIHS..... 55
PeGATA5 RRCTHCAVEE..TECWRLGPDGPRTLCNACGVRFKSGRLFPEYRPANSPTFSPLLHS..... 55
PeGATA6 KRCTHCMSYK..TECWRAGPLGPKTLCNACGVRFKSGRLLPEYRPANSPTFVSYMHS..... 55
PeGATA11 KRCTHCMSYK..TECWRAGPLGPKTLCNACGVRFKSGRLLPEYRPANSPTFVSYMHS..... 55
PeGATA31 RRCLHCETDK..TECWRTGPMGPKTLCNACGVRYKSGRLVQEYRPAASPTFMVSKHS..... 55
PeGATA10 MRCLHCETDR..TECWRTGPMGPKTLCNACGVRYKSGRLVPEYRPAASPTFMVSKHS..... 55
PeGATA30 RRCLHCETDK..TECWRTGPMGPKTLCNDAVQRVRGAVQVGAAGAGVPAVGEPDLRR..... 55
PeGATA17 RVCSDCNTTK..TELWRSGPCGPKAAEGAAGDDGLRG.GAKVGTPSDAATAHPKVKKE.... 55
PeGATA26 RVCSDCNTTK..TELWRSGPRGPKSLCNACGIRQRKA.RRAMMAS.GASTEGAKVGTPS... 55
PeGATA18 RVCSDCNTTK..TELWRSGPCGPKVKLLSRHPLSLIF.MCSMCNFASYRVLQVVTLIP.... 55
PeGATA24 RVCLDCNTTK..TELWRSGPCGPKSLCNACGIRQRKA.RRAMAAVTAAAANGGAAGVG.... 55
PeGATA28 RSCVECRTTT..TEMWRGGPTGPRSLCNACGIRYRKK.RRQELGQDQKQP.QQHRGEAT... 55
PeGATA29 TSCVECGTTT..TEMWRGGPTRPRSLCNACGIRYRKK.RRQELGLDQKQ..QQHHGEATT.. 55
PeGATA3 KACTDCHTTK..TELWRGGPSGPKSLCNACGIRYRKK.RREALGLDAGE..GAEQQQQKK.. 55
PeGATA16 KACTDCHTTK..TELWRGGPSGPKSLCNACGIRYRKK.RREALGLDAGE..GAEQQQKKK.. 55
PeGATA7 KACADCHTTK..TELWRGGPTGPKSLCNACGIRYRKR.RRQALGLDATETEGAEQQQQ.... 55
PeGATA13 KACADCHTTK..TELWRGGPTGPKSLCNACGIRYRKR.RRQALGLEAAA.EGAEQQQQK... 55
PeGATA27 RRCANCGTTS..TELWRNGPRGPKSLCNACGIRFKKEERRAAAAAESGGAWCGYSAQ..... 55
PeGATA8 RRCANCDTTS..TELWRNGPRGPKSLCNACGIRYKKEERRAAAAVAPPP..PQDSGVGY... 55
PeGATA12 NACANCDTTS..TELWRNGPRGPKSLCNACGIRYKKEERRAAAAAVAPTALPSDSGV..... 55
PeGATA22 IRCQNCGTSEKMTEAMRRGPAGPRTLCNACGLMWANKGT....LRSCPKANVEAPLVTI... 55
PeGATA23 LRCQNCGTSEKMTEAMRRGPAGPRTLCNACGLMWANKFLPYYFLWAWRKLFVDEQ..... 55
PeGATA4 SRCHHCGASATQTEMMRRGPDGPRTLCNACGLMWANKILVLEATSRCHHCGASAT..... 55
PeGATA15 SRCHHCGISATLTEMMRRGPDGPRMLCNACGLMWANKGMMRDLS.KAPTAPLRVVP..... 55
PeGATA14 .....THSRLTEAMRRGPTGPRSLCNACGLKWANKGTLRS.PLNAPKVTVQHPTNLSKMC 54
PeGATA20 TRCQNCGISSRLTEAMRRGPAGPRSLCNACGLMWANKGTLRS.PLNAPKMTLQHPA..... 55
Class A
Class B
Class C
GATA motif
bits
WebLogo 3.6.0

Fig.2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Subfamily Ⅱ
Subfamily Ⅲ
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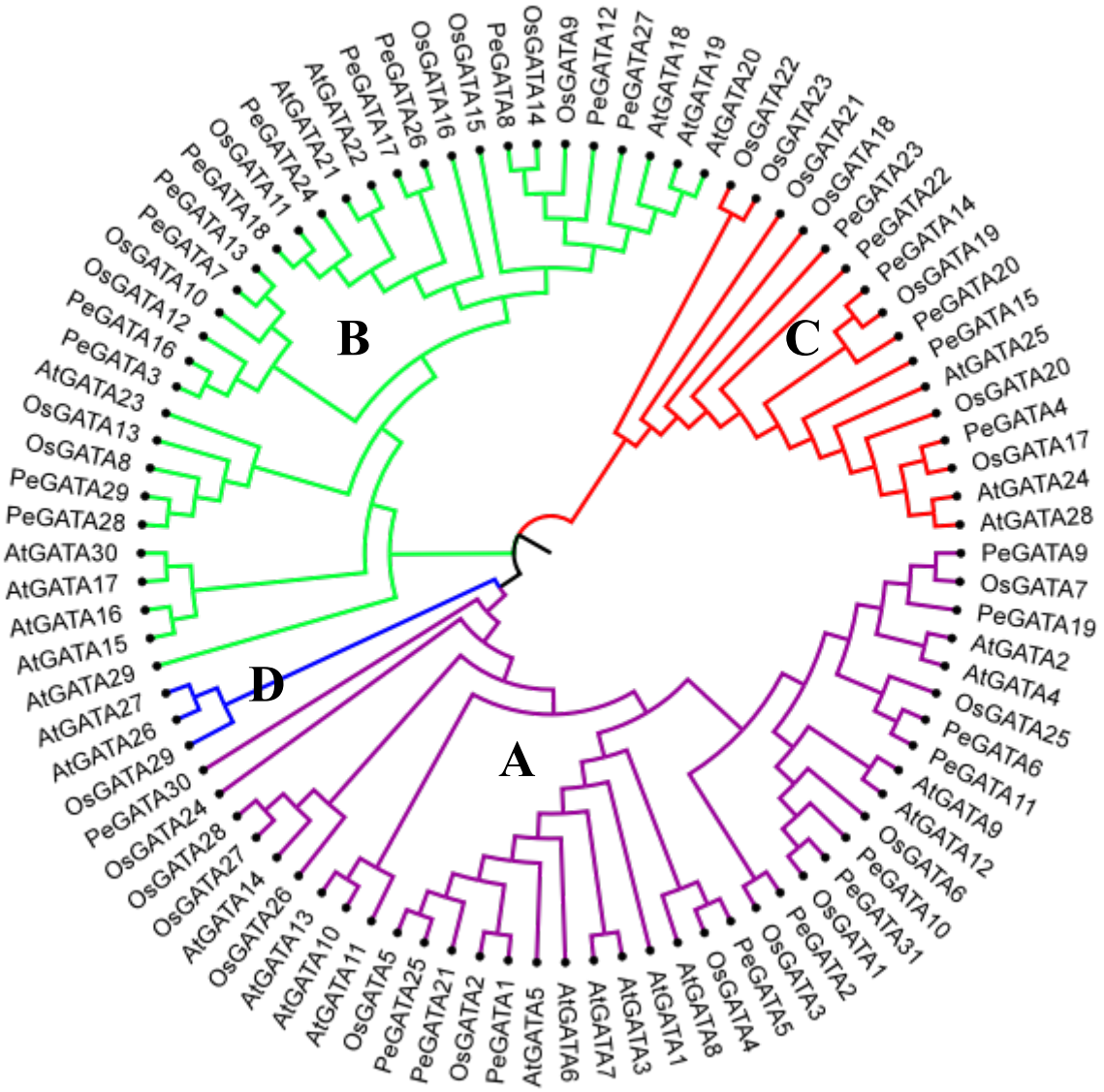
PeGATA9
PeGATA19
PeGATA2
PeGATA5
PeGATA6
PeGATA11
PeGATA31
PeGATA10
PeGATA30
PeGATA17
PeGATA26
PeGATA18
PeGATA24
PeGATA28
PeGATA29
PeGATA3
PeGATA16
PeGATA7
PeGATA13
PeGATA27
PeGATA8
PeGATA12
PeGATA22
PeGATA23
PeGATA4
PeGATA15
PeGATA14
PeGATA20
5'
3'
0
100
200
300
400
500
Motif 10
Motif 5
Motif 1
Motif 2
Motif 7
Motif 6
Motif 8
Motif 4
Motif 9
Motif 3
ZnF_GATA
PLN00062
ZnF_GATA superfamily
GATA
CCT
tify
IncA superfamily

Fig.3


A
B
C
D
OsGATA9
PeGATA12
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OsGATA21
OsGATA18
PeGATA23
PeGATA22
PeGATA14
PeGATA19
OsGATA19
PeGATA20
PeGATA15
AtGATA25
OsGATA20
PeGATA4
OsGATA17
AtGATA24
AtGATA28
PeGATA9
OsGATA7
PeGATA19
AtGATA2
AtGATA4
OsGATA25
PeGATA6
PeGATA11
AtGATA9
AtGATA12
OsGATA6
PeGATA10
PeGATA31
OsGATA1
PeGATA2
OsGATA3
PeGATA5
OsGATA4
AtGATA8
AtGATA1
AtGATA3
AtGATA7
AtGATA6
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AtGATA13
OsGATA26
AtGATA14
OsGATA27
OsGATA28
PeGATA24
OsGATA29
PeGATA30
AtGATA26
AtGATA27
AtGATA29
AtGATA15
AtGATA16
AtGATA17
AtGATA30
PeGATA28
PeGATA29
OsGATA8
OsGATA13
AtGATA23
PeGATA3
PeGATA16
OsGATA12
OsGATA10
PeGATA7
PeGATA13
PeGATA18
OsGATA11
PeGATA24
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AtGATA22
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PeGATA26
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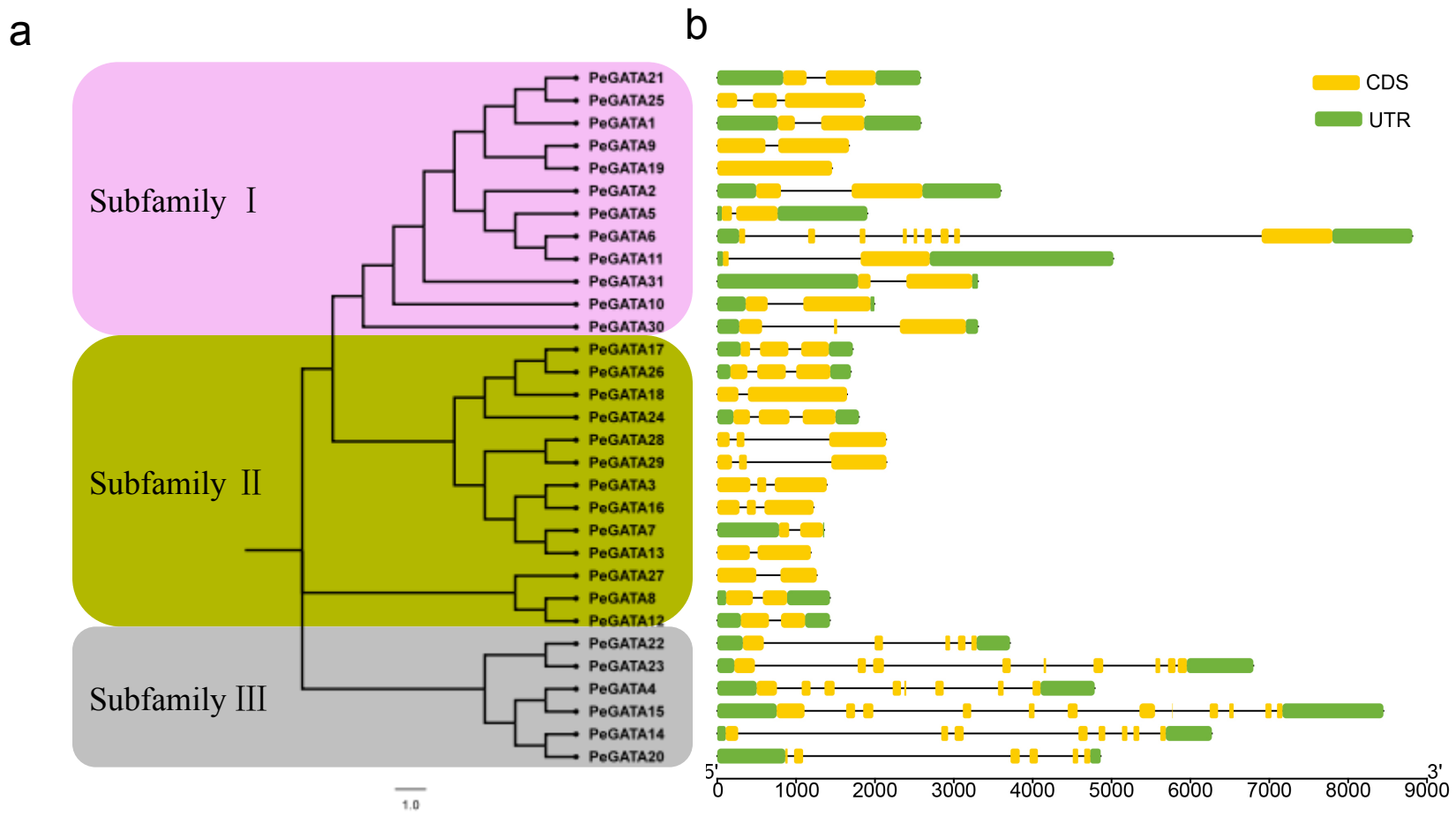
OsGATA15
PeGATA8
OsGATA14

Fig.4


a
b
Subfamily Ⅰ
Subfamily Ⅱ
Subfamily Ⅲ
PeGATA21
PeGATA25
PeGATA1
PeGATA9
PeGATA19
PeGATA2
PeGATA5
PeGATA6
PeGATA11
PeGATA31
PeGATA10
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PeGATA28
PeGATA29
PeGATA3
PeGATA16
PeGATA7
PeGATA13
PeGATA27
PeGATA8
PeGATA12
PeGATA22
PeGATA23
PeGATA4
PeGATA15
PeGATA14
PeGATA20
1.0
CDS
UTR
5'
3'
0
1000
2000
3000
4000
5000
6000
7000
8000
9000

Fig.5

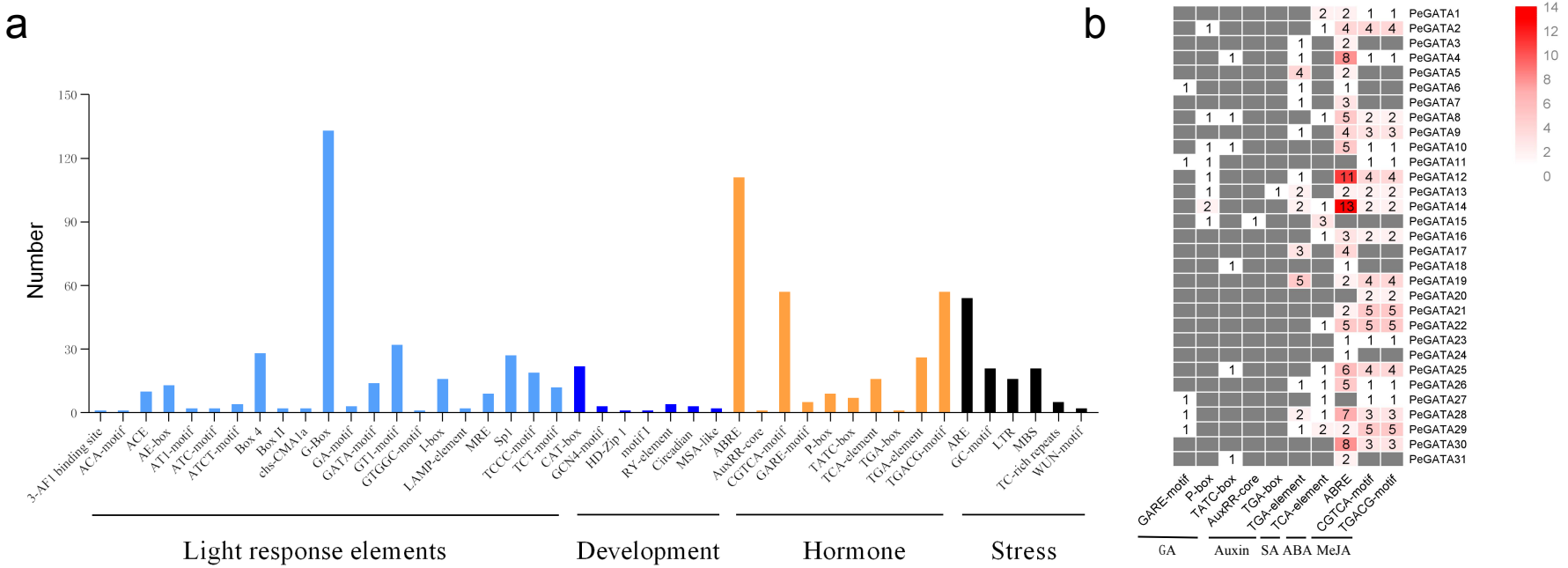

a
Number
150
120
90
60
30
0
3-AF1 binding site
ACA-motif
ACE
AE-box
ATI-motif
ATC-motif
ATCT-motif
Box 4
Box II
chs-CMA1a
G-Box
GA-motif
GATA-motif
GT1-motif
GTGGC-motif
I-box
LAMP-element
MRE
Sp1
TCCC-motif
TCT-motif
CAT-box
GCN4-motif
HD-Zip 1
motif1
RY-element
Circadian
MSA-like
ABRE
AuxRR-core
CGTCA-motif
GARE-motif
P-box
TATC-box
TCA-element
TGA-box
TGA-element
TGACG-motif
ARE
GC-motif
LTR
MBS
TC-rich repeats
WUN-motif
Light response elements
Development
Hormone
Stress
b
PeGATA1
PeGATA2
PeGATA3
PeGATA4
PeGATA5
PeGATA6
PeGATA7
PeGATA8
PeGATA9
PeGATA10
PeGATA11
PeGATA12
PeGATA13
PeGATA14
PeGATA15
PeGATA16
PeGATA17
PeGATA18
PeGATA19
PeGATA20
PeGATA21
PeGATA22
PeGATA23
PeGATA24
PeGATA25
PeGATA26
PeGATA27
PeGATA28
PeGATA29
PeGATA30
PeGATA31
GARE-motif
P-box
TATC-box
AuxRR-core
TGA-box
TGA-element
TCA-element
ABRE
CGTCA-motif
TGACG-motif
GA
Auxin
SA
ABA
MeJA
14
12
10
8
6
4
2
0

Fig.6

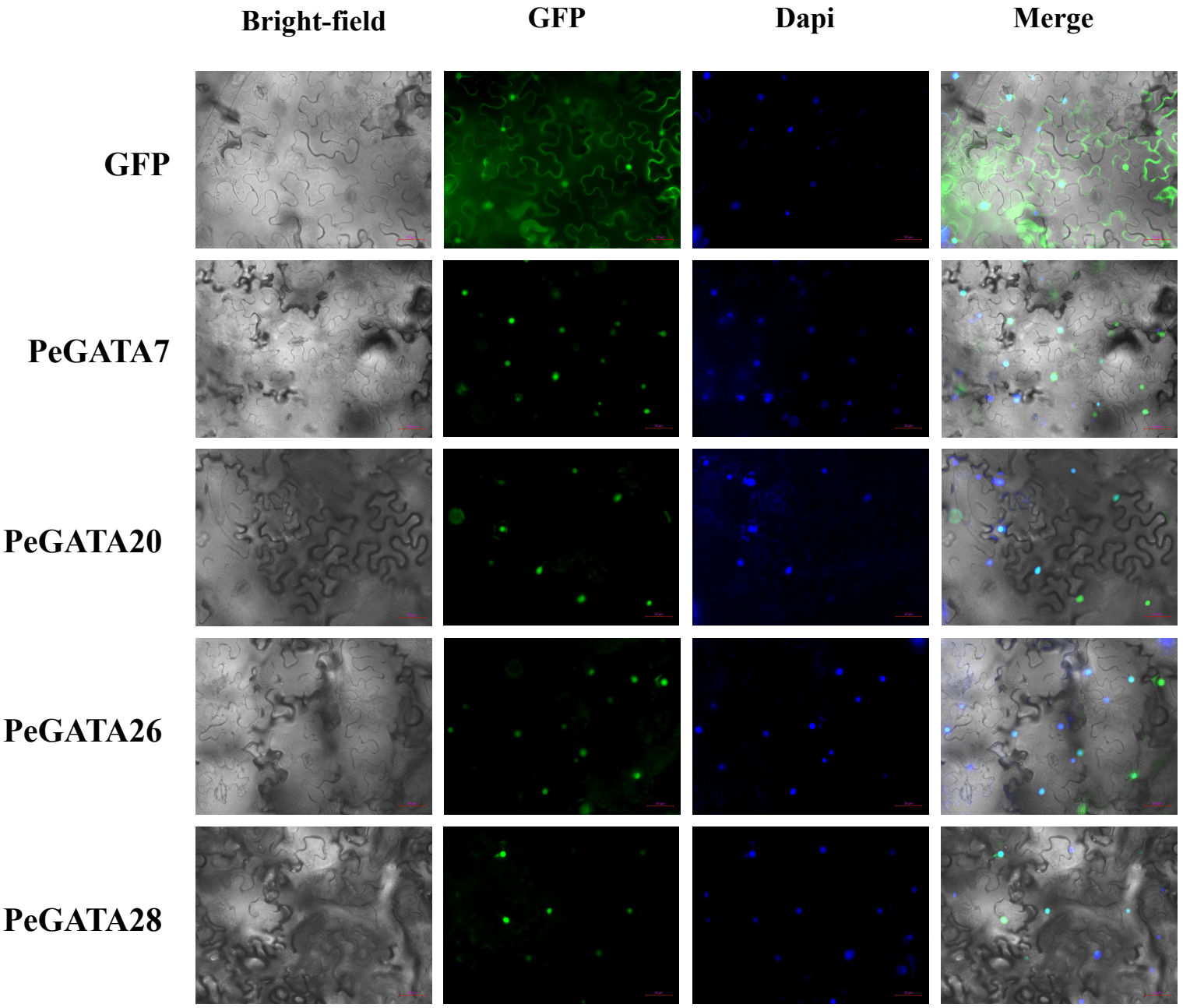
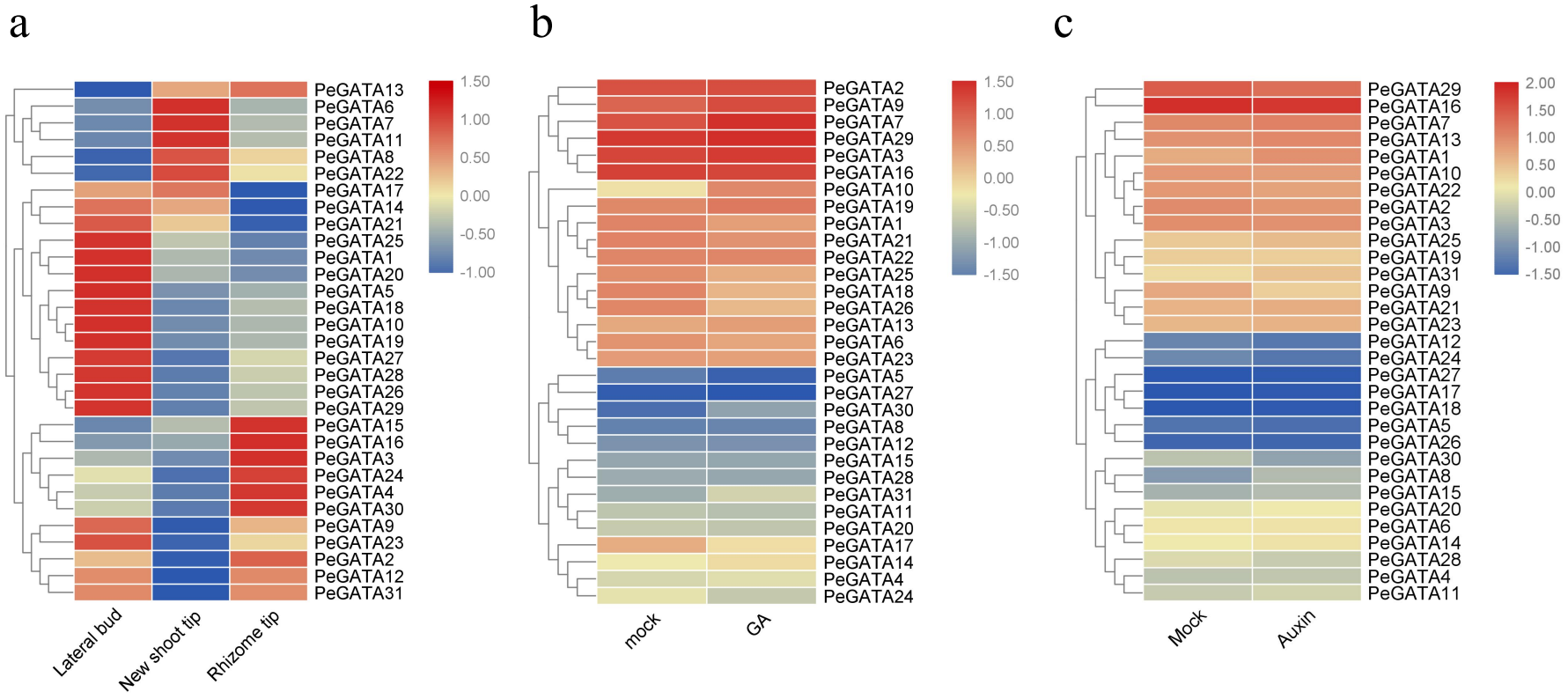

Bright-field
GFP
Dapi
Merge
GFP
PeGATA7
PeGATA20
PeGATA26
PeGATA28

Fig.7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PeGATA8
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PeGATA14
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PeGATA25
PeGATA1
PeGATA20
PeGATA5
PeGATA18
PeGATA10
PeGATA19
PeGATA27
PeGATA28
PeGATA26
PeGATA29
PeGATA15
PeGATA16
PeGATA3
PeGATA24
PeGATA4
PeGATA30
PeGATA9
PeGATA23
PeGATA2
PeGATA12
PeGATA31
1.50
1.00
0.50
0.00
-0.50
-1.00
Lateral bud
New shoot tip
Rhizome tip
b
PeGATA2
PeGATA9
PeGATA7
PeGATA29
PeGATA3
PeGATA16
PeGATA10
PeGATA19
PeGATA1
PeGATA21
PeGATA22
PeGATA25
PeGATA18
PeGATA26
PeGATA13
PeGATA6
PeGATA23
PeGATA5
PeGATA27
PeGATA30
PeGATA8
PeGATA12
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PeGATA28
PeGATA31
PeGATA11
PeGATA20
PeGATA17
PeGATA14
PeGATA4
PeGATA24
1.50
1.00
0.50
0.00
-0.50
-1.00
-1.50
mock
GA
c
PeGATA29
PeGATA16
PeGATA7
PeGATA13
PeGATA1
PeGATA10
PeGATA22
PeGATA2
PeGATA3
PeGATA25
PeGATA19
PeGATA31
PeGATA9
PeGATA21
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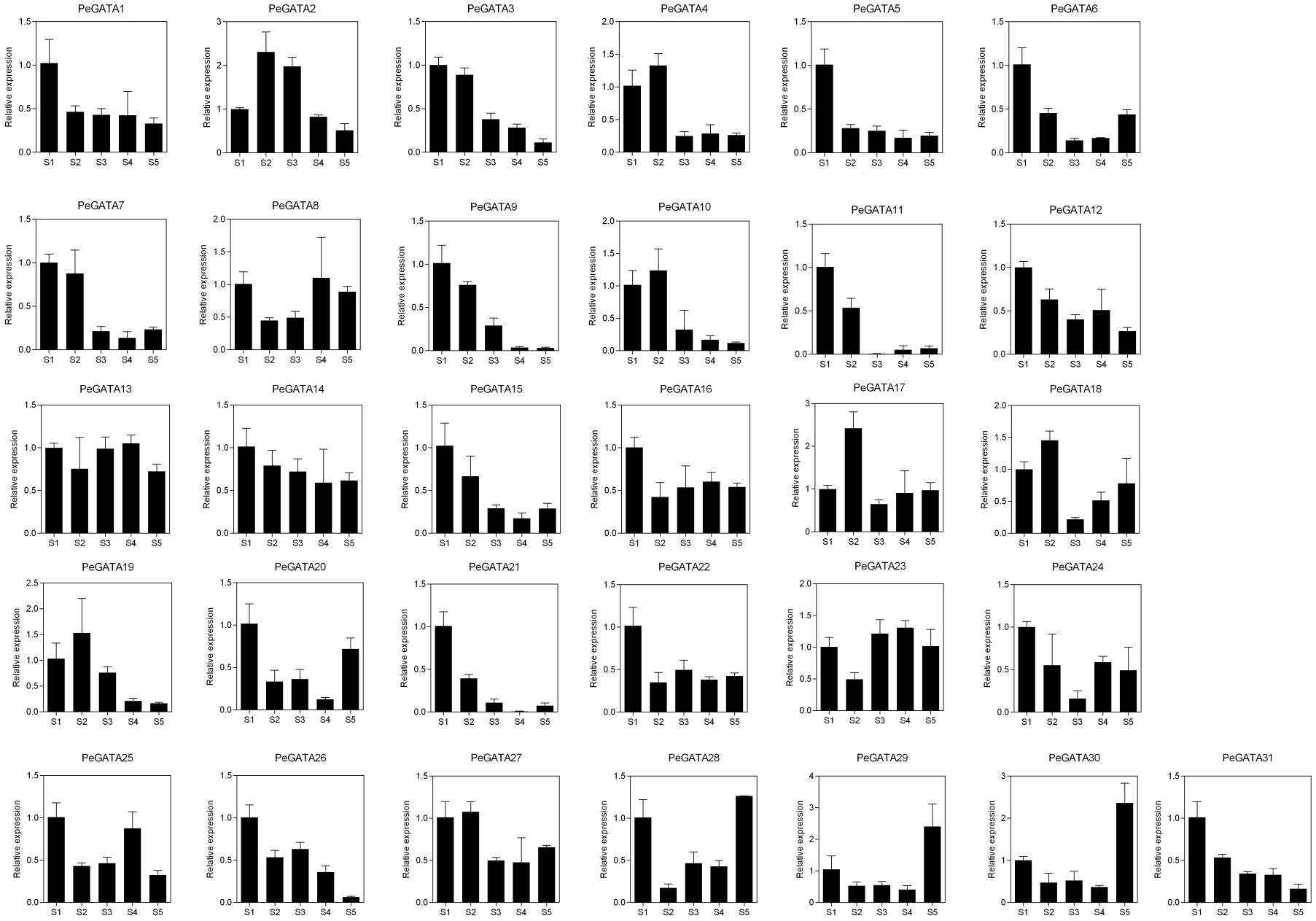
PeGATA12
PeGATA24
PeGATA27
PeGATA17
PeGATA18
PeGATA5
PeGATA26
PeGATA30
PeGATA8
PeGATA15
PeGATA20
PeGATA6
PeGATA14
PeGATA28
PeGATA4
PeGATA11
2.00
1.50
1.00
0.50
0.00
-0.50
-1.00
-1.50
Mock
Auxin

Fig.8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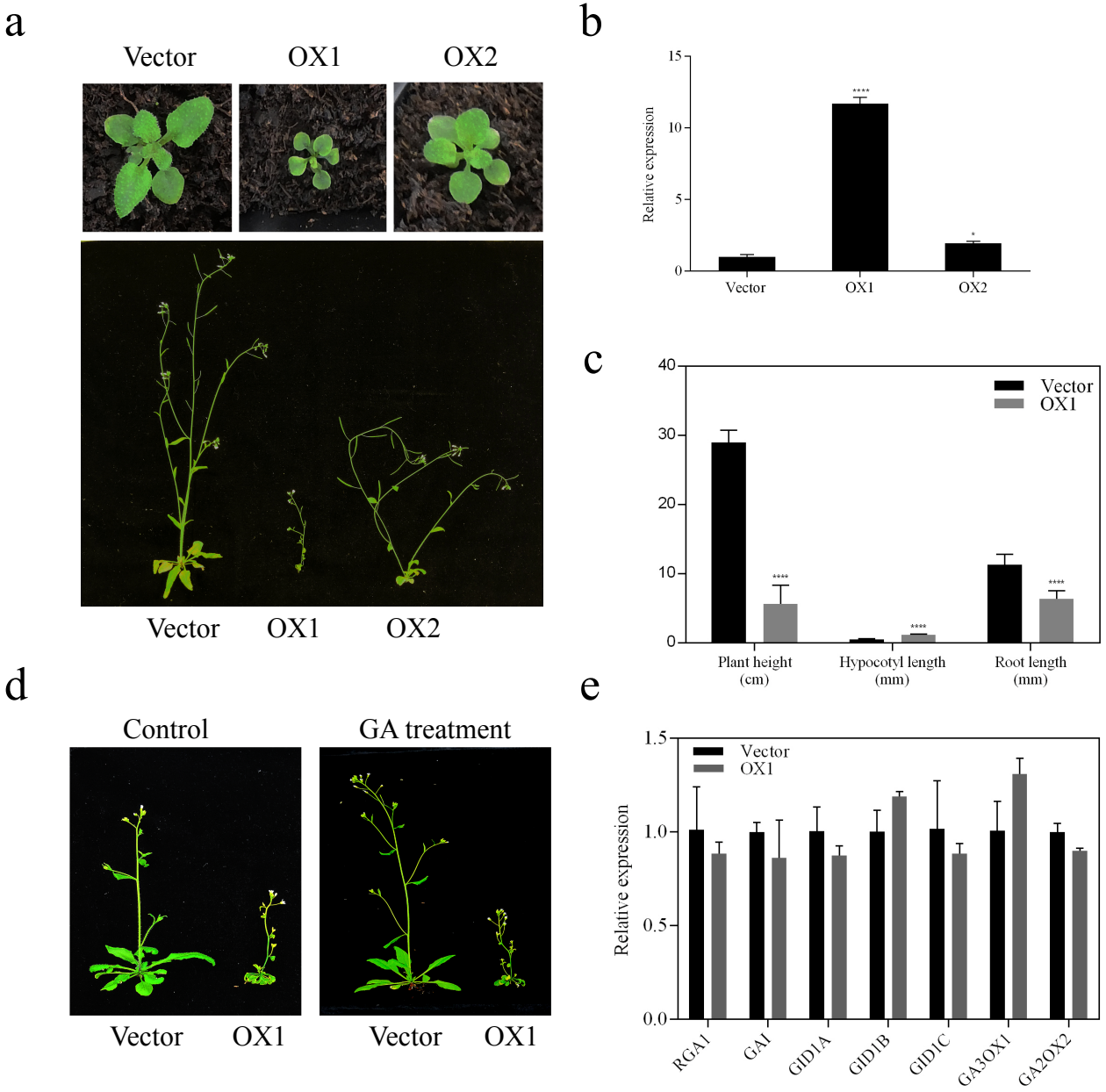
PeGATA3
PeGATA4
PeGATA5
PeGATA6
PeGATA7
PeGATA8
PeGATA9
PeGATA10
PeGATA11
PeGATA12
PeGATA13
PeGATA14
PeGATA15
PeGATA16
PeGATA17
PeGATA18
PeGATA19
PeGATA20
PeGATA21
PeGATA22
PeGATA23
PeGATA24
PeGATA25
PeGATA26
PeGATA27
PeGATA28
PeGATA29
PeGATA30
PeGATA31
Relative expression
S1
S2
S3
S4
S5

Fig.9


a
Vector
OX1
OX2
Vector
OX1
OX2
b
Relative expression
15
10
5
0
****
*
Vector
OX1
OX2
c
40
30
20
10
0
Vector
OX1
****
****
****
Plant height (cm)
Hypocotyl length (mm)
Root length (mm)
d
Control
GA treatment
Vector
OX1
Vector
OX1
e
Relative expression
1.5
1.0
0.5
0.0
Vector
OX1
RGA1
GAI
GID1A
GID1B
GID1C
GA3OX1
GA2OX2