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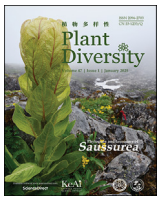

Research paper

Subgenome asymmetry of gibberellins-related genes plays important roles in regulating rapid growth of bamboos

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ABSTRACT

Rapid growth is an innovative trait of woody bamboos that has been widely studied. However, the genetic basis and evolution of this trait are poorly understood. Taking advantage of genomic resources of 11 representative bamboos at different ploidal levels, we integrated morphological, physiological, and transcriptomic datasets to investigate rapid growth. In particular, these bamboos include two large-sized and a small-sized woody species, compared with a diploid herbaceous species. Our results showed that gibberellin A1 was important for the rapid shoot growth of the world's largest bamboo, *Dendrocalamus sinicus*, and indicated that two gibberellins (GAs)-related genes, *KAO* and *SLRL1*, were key to the rapid shoot growth and culm size in woody bamboos. The expression of GAs-related genes exhibited significant subgenome asymmetry with subgenomes A and C demonstrating expression dominance in the large-sized woody bamboos while the generally submissive subgenomes B and D dominating in the small-sized species. The subgenome asymmetry was found to be correlated with the subgenome-specific gene structure, particularly UTRs and core promoters. Our study provides novel insights into the molecular mechanism and evolution of rapid shoot growth following allopolyploidization in woody bamboos, particularly via subgenome asymmetry. These findings are helpful for understanding of how polyploidization in general and subgenome asymmetry in particular contributed to the origin of innovative traits in plants.

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1. Introduction

The rapid growth of plants holds substantial importance, which helps plants to occupy their ecological niche, adapt to varying environmental conditions, and can also generate economic value for human beings (Rubin, 2008). In recent years, cultivation of fast-growing plants has emerged as a possible way to meet the increasing global demand for renewable biomass. For example, paulownias timber can be harvested in six years (Cao et al., 2021) with the *Eucalyptus* species at even a younger age (Zhang and Wang, 2021). Polars can grow very fast under suitable conditions (Bunder et al., 2020), enabling efficient and sufficient timber supply. In practice, the rapid growth and size regulation of crops have a profound impact on agricultural performance and productivity (Sasaki and Ashikari, 2018).

Previous studies suggested that rapid growth and size regulation in plants is affected by several factors including cell proliferation, cell elongation and phytohormones (Zhang and Wang, 2021; Chen et al., 2022). In particular, a large number of genes in controlling plant size have been identified in cereals (e.g., rice, wheat, barley) (Sasaki and Ashikari, 2018) and *Arabidopsis thaliana* (Regnault et al., 2014), and most of them functioning in the biosynthesis or signal transduction of gibberellins (GAs). Phytohormones, particularly GAs, also play a significant role in culm growth and internode elongation of moso bamboo, *Phyllostachys edulis* (Tao et al., 2018). The previous studies mainly focused on *P. edulis* showed that GAs and auxin (IAA) content were positively correlated with the growth rate, while cell division rate during the internode elongation was positively correlated with zeatin content and negatively correlated with abscisic acid (ABA) content (Ding, 1997; Cui et al., 2012; Chen et al., 2022). The application of exogenous GAs (GA3 and GA4) on *P. edulis* seedlings led to a significant increase in internode length, culm size, and shoot growth rate (Zhang et al., 2018; Chen et al., 2022).

Belonging to the grass family (Poaceae), the bamboos (Bambusoideae) exhibit a high degree of diversity and possess many unique traits compared to other grasses (Liese and Köhl, 2015). The unique traits in woody bamboos include rapid shoot growth, highly lignified mature culms, and massive and synchronous flowering (Wang et al., 2024b), which are absent in herbaceous bamboos. The distinct traits make woody bamboos as important non-timber forest plants due to their ecological, and economic value, as they are extensively distributed across subtropical and tropical regions (Liese and Köhl, 2015). To date, researchers have documented approximately 1700 species (Soreng et al., 2022) of bamboos into one minor diploid herbaceous lineage (HBs, $2n = 2x$, 20–24, HH) and three major polyploid woody lineages (Guo et al., 2019). These woody lineages encompass tetraploid temperate woody bamboos (TWBs, $2n = 4x = 46–48$, CCDD), tetraploid neotropical woody bamboos (NWBs, $2n = 4x = 40–48$, BBCC), and hexaploid paleotropical woody bamboos (PWBs, $2n = 6x = 70–72$, AABBCC) (Guo et al., 2019; Ma et al., 2024). Although some PWBs may bear higher chromosome number ($2n = 9x = 104$), it was revealed that they possessed three haplotype genomes, each including the subgenomes A, B, and C (Wang et al., 2024b). The bamboos represent an exceptional polyploid system and have undergone complex reticulate evolution with multiple hybridizations and polyploidizations, which may trigger widespread species diversification due to adaptation (Alger and Edger, 2020; Ma et al., 2024). The subgenomes from different progenitors can regulate traits asymmetrically, for instance, in allotetraploid cotton *Gossypium hirsutum*, fiber quality-related homoeologs show phenotypically favorable allele aggregation in different cultivars (You et al., 2023), and salt responsiveness in both *Go. hirsutum* (AD1) and *Go. mustelinum* (AD4) was strongly biased toward the D-genome progenitor (Dong et al., 2022). However, a recent study in *Nepenthes gracilis* illustrated that recessive subgenomes contribute most to the adaptive evolution of tissue-specific genes (Saul et al., 2023).

Woody bamboos as an essential alternative to timber are important sources of lignocellulosic biomass energy (Liese and Köhl, 2015). Previous studies indicated that growth of bamboo shoots exhibit a “slow-fast-slow” pattern (Chen et al., 2022; Niu et al., 2022). Moreover, the fastest growth rate of bamboo shoots was suggested to be positively correlated to culm size of bamboos, and that this was achieved through the simultaneous growth of multiple culm internodes (Wang et al., 2012a; Huang et al., 2016; Tan and Wu, 2018; Xu, 2018). In recent years, high-throughput sequencing technology has been extensively employed in the investigation of the rapid growth mechanisms in woody bamboos. Through transcriptome and proteome analyses, numerous differentially expressed genes (DEGs) were identified to be related to phytohormone biosynthesis and signal transduction, lignin and cellulose biosynthesis, carbohydrate metabolism and key transcription factors (Cui et al., 2012; Peng et al., 2013; Wei et al., 2018). For example, the *Snf1* (Wei et al., 2019) and *COMT* genes may have an effect on the lignin content of woody bamboos (Ma et al., 2024), while ABA and mechanical pressure stimulate rapid secondary cell wall (SCW) thickening by upregulating the MYB transcription factor *PeMYB83L* in *P. edulis* (Chen et al., 2022). The microRNAs (miRNAs)-mediated regulatory pathways also play a vital role in rapid growth of *P. edulis* (Wang et al., 2021a; Yang et al., 2021). Furthermore, DNA methylation could also play a critical role in the regulation of the rapid shoot growth and culm development in *Bonia amplexicaulis* and *P. edulis*, and the nanopore direct RNA sequencing (DRS) revealed a dynamic N6-methyladenine (m6A) modification rate in shoots of different height (Niu et al., 2022; Li et al., 2023). And the new genes and whole-genome duplicates have underlined the genetic basis for the evolving rapid growth of woody bamboos (Jin et al., 2021).

In addition, the auxin and cytokinin signaling crosstalk was suggested to regulate bamboo growth by *PheARF52* and *PheRR3* (Bai et al., 2023), and auxin signaling could directly modulate lignin biosynthesis genes to alter culm lignin content through auxin response factors *ARF3* and *ARF6* during shoot development (Wang et al., 2024a). By analyzing extensive interspecific culm variations among 77 woody bamboo species, researchers found the heritable legacy playing a key role in the regulation of functional traits of culms and the climate acting as a relative moderating factor (Liu et al., 2024b). The internode diameter, thickness, length, and volume were correlated to one another and the precipitation may affect the primary thickening growth and, consequently, the culm size (Zhang et al., 2024). And the decreased woody bamboo internode length may be due to a decrease in cell number and cell length (Zha et al., 2023). Spatiotemporal transcriptome atlas supports the hypotheses that the intercalary meristem originates from surrounded parenchyma cells through dedifferentiation (Guo et al., 2024). Taken together, the extant studies on rapid growth of bamboos have mainly focused on a few economically important bamboos, particularly the tetraploid *P. edulis*. Notably, woody bamboos show a great of diversity with culm size. For example, the height of *Shibataea chiangshanensis* is only 0.5 m, while that of *P. edulis* can reach 20 m (Shi et al., 2021). The largest known bamboo, hexaploid *Dendrocalamus sinicus* can reach 37.5 m in height with culm diameter to 28.7 cm (Liu et al., 2024a; Ma et al., 2024).

The woody bamboos have undergone multiple whole-genome duplications (WGDs), as well as small-scale duplications (Ma et al., 2024). These events would lead to the divergence of gene across different tissues through processes such as neo- or subfunctionalization (Guo et al., 2024). Built on our previous work on

chromosome-level genome sequences of 11 representative bamboos (Ma et al., 2024), the current study aimed to further investigate the molecular mechanism and evolution of rapid shoot growth in woody bamboos. Importantly, the 11 representative bamboos covered different ploidal levels, genome and culm size diversity. And we adopted an integrated approach with morphological, physiological, transcriptomic and genomic analyses to address the questions.

The height of well-growing woody bamboos and the growth patterns of key bamboos were determined, and levels of representative phytohormones were measured in rapid growing shoots. Comprehensive transcriptomes of different bamboo species were analyzed with key candidate genes regulating the culm growth and internode elongation. We also investigated the expression patterns of those genes in representative bamboos using available bamboo genome and transcriptome data, and compared the gene structure of key genes located in different subgenomes in individual bamboo species. Our study aims to determine the genetic basis of rapid shoot growth in the context of species diversification following hybridizations and polyploidizations of woody bamboos.

2. Materials and methods

2.1. Morphological analysis of representative woody bamboos

To investigate the differences in culm size among woody bamboos, we selected well-growing population of seven representative woody bamboos for measurements. Including three tetraploid TWBs (*Ampelocalamus luodianensis*, *Hsuehochloa calcarea* and *P. edulis*), one tetraploid NWB (*Guadua angustifolia*), and three hexaploid PWBs (*Melocanna baccifera*, *B. amplexicaulis* and *D. sinicus*).

2.2. Investigation of the growth pattern of shoots and internodes of Phyllostachys edulis

We continuously measured the shoot height and 9th, 10th, 11th, 12th internode length of young shoots of *P. edulis* until the end of the target internode elongation in April to May 2021 at Zhongchang Village, Yiliang County, Yunnan Province, China (27°53′35″N, 104°42′59″E, alt. 1120 m). A total of 115 well-growing shoots was selected, with the smallest one being 5 cm.

2.3. Quantification of phytohormones in Dendrocalamus sinicus internodes during rapid shoot growth

The samples of shoots used to quantify contents was consistent with transcriptome analyses and anatomical observation in our previous work (Ma et al., 2024). The contents of endogenous IAA, ABA, and cytokinin (CK) were determined using previously published methods with slight modifications (Cao et al., 2021; Song et al., 2022), the levels of GAs were measured by the previously described method (Liu et al., 2019).

2.4. Plant material and transcriptome sequencing

The culms of the herbaceous bamboo, *Raddia guianensis* were divided into three stages on the basis of different developmental statuses (Fig. S1). We peeled off leaves and leaf sheaths, then culms were collected for transcriptome sequencing and analyses. The sequencing and analyses methods followed previous studies (Tao et al., 2020; Cao et al., 2021; Yang et al., 2021; Chen et al., 2022; Niu et al., 2022).

2.5. Transcriptome analyses

Transcriptome sequence processing, assembly, and quantification of *Ra. guianensis* culms were performed using methods described (Ma et al., 2024). All of the quantification results above were used to do the clustering analysis among samples in species, and the checked samples were kept for subsequent analyses. Then, DEGs during rapid shoot growth in three woody bamboos (*D. sinicus* as a large-sized PWB, *P. edulis* as a large-sized TWB, and *B. amplexicaulis* as a small-sized PWB) and regular growth in a herbaceous bamboo *Ra. guianensis* at different developmental stages and tissues were identified. After that, weighted correlation network analysis of DEGs in *D. sinicus*, *P. edulis*, and *B. amplexicaulis* were performed with WGCNA (Langfelder and Horvath, 2008), as well as KEGG and GO enrichment were performed in parallel. And gene interaction networks in important modules were visualized using Cytoscape (Shannon et al., 2003).

2.6. Identification of homoeolog genes in representative bamboos

In order to further investigate the molecular mechanism of rapid shoot growth in woody bamboos, we identified the gene copies related to GAs in 11 bamboo species. We used known genes involved in GAs biosynthesis and signal transduction pathway (https://rapdb.dna.affrc.go.jp/) from *Oryza sativa* as seed sequence to identify homologous gene copies in two herbaceous species (HBs, *Olyra latifolia* and *Ra. guianensis*), and nine woody bamboos: TWBs (*Am. luodianensis*, *H. calcarea* and *P. edulis*), NWBs (*Rhipidocladum racemiflorum*, *Otatea glauca* and *Gu. angustifolia*) and PWBs (*M. baccifera*, *B. amplexicaulis* and *D. sinicus*). The protein sequences of *O. sativa* (http://www.genobank.org/grass) and 11 bamboo genomes (https://bamboo.genobank.org/) were downloaded, and the GAs-related genes in *O. sativa* was extracted for seed sequences. Identification methods were described in our previous work (Ma et al., 2024). Moreover, MCScan was used with default parameters to identify the homoeologs among bamboo subgenomes and rice genome (Wang et al., 2012b).

2.7. Gene expression analysis

We further investigated the expression patterns of homoeolog genes involved in GAs during rapid shoot growth in eight woody bamboos with the exclusion of *Rh. racemiflorum* due to the lack of related shoot samplings, the transcripts per million (TPM) value of shoots at different developmental stages in woody bamboos were employed for gene expression analysis (Ma et al., 2024).

Based on the identified GAs-related DEGs during rapid growth, we further integrated the transcriptome data from young shoot of eight representative woody bamboos to check the expression patterns of GA-related genes among species and subgenomes. In *D. sinicus*, *P. edulis* and *B. amplexicaulis*, we defined the DEGs identified from their own transcriptome data during rapid shoot growth. The remaining bamboo species without rapid growing shoot transcriptome data were calculated using homologous genes that were identified as DEGs in the three species. Initially, we compared the expression proportion of GAs-related DEGs in total GAs-related genes within young shoots across eight woody bamboos with different culm sizes, and obtained the expression patterns of GAs-related DEGs across different woody bamboos. Furthermore, we analyzed the expression levels of GA-related DEGs on different subgenomes in individual bamboo species, including rapid growing shoots of *D. sinicus*, *P. edulis* and *B. amplexicaulis* and young shoots of eight woody bamboos (except *Rh. racemiflorum*). Through this process, we were able to identify the expression

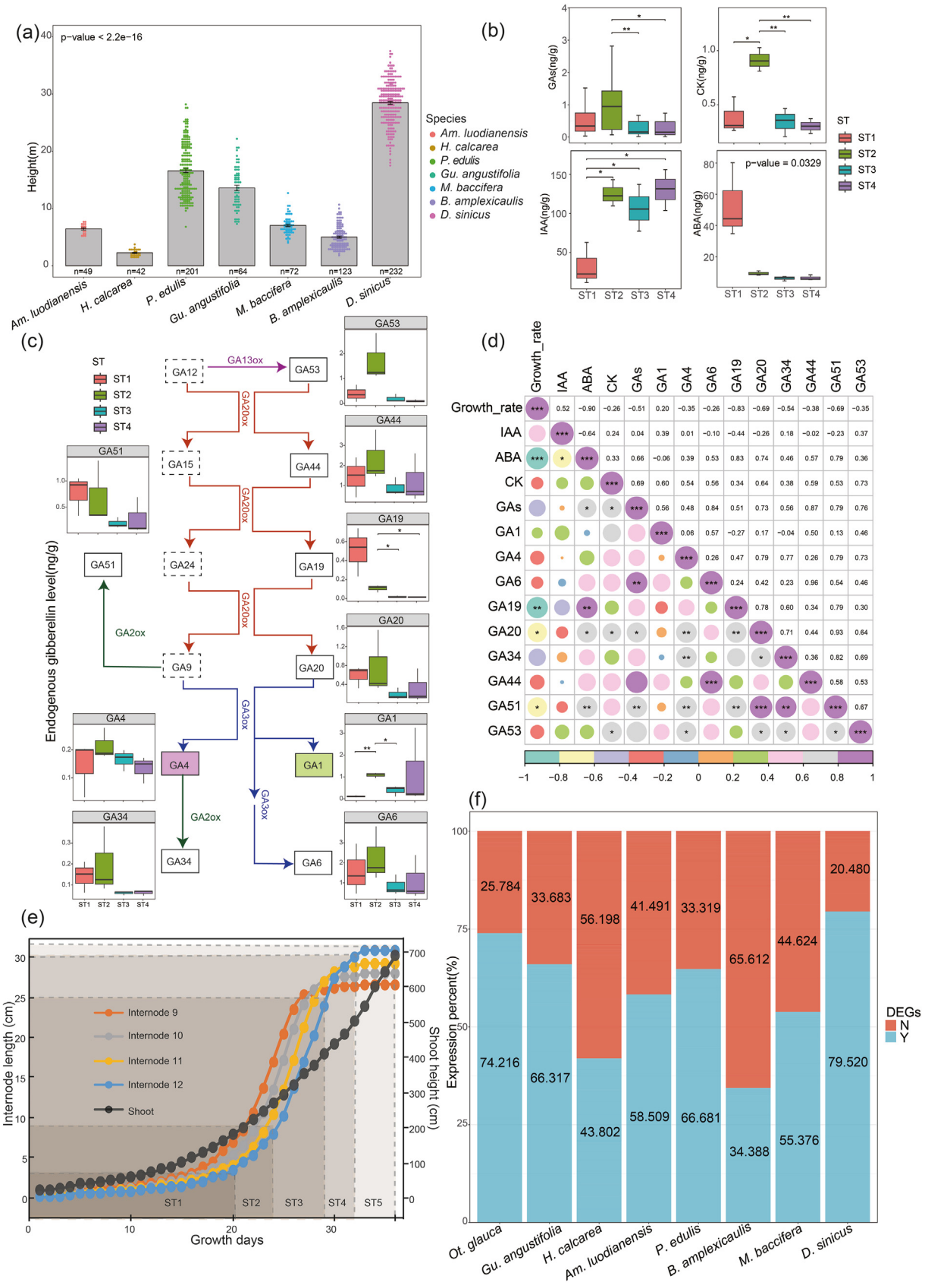

Fig. 1. GAs was important in bamboos with different culm sizes. (a) Height divergence of seven representative woody bamboos. The height of different woody bamboos varies significantly (p-value < 2.2e −16, Kruskal test). The number of mature bamboo culms measured (n) is shown. (b) Quantitation of endogenous phytohormone contents of internodes during rapid growth in *Dendrocalamus sinicus*. GAs: content of total gibberellins, CK: content of cytokinin, IAA: content of auxin, ABA: content of abscisic acid. Data are mean ± s.d. (n = 3 shoots). Asterisks and p-value = 0.0329 represent the content of shoots among/between different stages were varies significantly (Kruskal test). (c) Biosynthesis and contents

patterns of key GA-related genes across different subgenomes, and the results were exhibited using R package.

2.8. Gene structure analyses of hub genes involved in GAs

To study structure differences of hub genes involved in GAs in bamboos, phylogenetic trees were constructed by IQ-TREE 2 with the sequence alignment trimmed as described in our previous work (Ma et al., 2024). The gene structures of hub genes were analyzed using TBtools (Chen et al., 2020a). The Multiple Expectation Maximization for Motif Elicitation (http://meme-suite.org/tools/meme) program was used to identify conserved motifs using multiple alignment analysis with default parameters. And the domains of the genes were identified using the NCBI Conserved Domain Database (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi). To identify the cis-acting elements, we extracted the 2000 bp upstream sequences of the transcription start site of the genes and the cis-acting elements(CREs) in the putative promoter regions were predicted using PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/). The results were visualized by R package and TBtools (Chen et al., 2020a).

3. Results

3.1. Morphological observation of representative woody bamboos

To explore the variation in culm size among woody bamboos with differing ploidal levels and genome diversity, we measured the height of seven representative woody bamboos for a total of 783 mature culms (Fig. 1a). Results showed that *D. sinicus* was the largest bamboo, followed by *P. edulis*. Notably, the culm size of bamboos with identical ploidy also varied widely. Among PWBs, the mean height of *D. sinicus* (28.4 m) and *B. amplexicaulis* (5.0 m) was in sharp contrast, and this phenomenon was also observed in different tetraploid TWBs (1.4 m in *H. calcarea* versus 16.6 m in *P. edulis*), indicating that in addition to ploidy (Sattler et al., 2016), there are more complex regulation mechanisms affecting culm growth and size remained to be explored.

After measuring heights of different woody bamboos, we focused on the hexaploid *D. sinicus* and *B. amplexicaulis*, both of PWBs which have consistent subgenomes composition but contrasting in culm size, as well as *P. edulis* as one of the largest in tetraploid bamboos. To accurately perform the rapid growth transcriptome sampling, we investigated the growth pattern of shoots and internodes in *P. edulis* as described in the *D. sinicus* and *B. amplexicaulis* (Niu et al., 2022; Ma et al., 2024). There was a similar shoot growth pattern of *P. edulis* with other woody bamboos (Wang et al., 2012a; Tan and Wu, 2018; Xu, 2018). The shoot growth stages for *P. edulis* followed a similar pattern as in *B. amplexicaulis* (Niu et al., 2022) and *D. sinicus* (Ma et al., 2024). Parallel to *B. amplexicaulis* and *D. sinicus*, we selected the 10th internode for analysis on *P. edulis*. When the shoot height is less than 50 cm, the growth rate of the shoot is notably slow, with daily height growth less than 5 cm; this period is referred to as the dormant stage (ST1). Subsequently, the growth rate of the shoot gradually increases with the height of 150 cm and we refer to this period as the initial rapid growth stage (ST2). Following that, both the shoot growth and the internode elongation are further accelerated. When the shoot height reaches 550 cm and the length of the internode is approximately 25 cm, it is defined as the rapid growth stage (ST3). Afterward, the elongation rate of the internode decelerates when the shoot height is about 650 cm; this is the end of rapid growth stage (ST4). Finally, the internode elongation ceases when reaching about 27 cm, termed as the mature stage (ST5).

3.2. Phytohormone changes in internode of Dendrocalamus sinicus during rapid shoot growth

Hormonal regulation was recognized as a key process in plant growth and development, and we quantified content of phytohormones (ABA, IAA, GAs, CK) in the 10th internode of *D. sinicus* shoots at different growth stages of ST1 to ST4. Measurements of total GAs showed that the content of total GAs in the ST2 stage was higher than other stages (especially ST3 and ST4). Moreover, the content of CK in the ST2 stage was also the highest (Fig. 1b). The content of auxin (IAA) in the ST1 stage was significantly lower than other stages, which was consistent with recent studies in *P. edulis* (Tao et al., 2018). In contrast to IAA, the ABA content in ST1 was significantly higher than the remaining stages, which is a well-known growth inhibitor by inhibiting cell division, elongation and metabolism (Chen et al., 2020b). These results indicated that cell division and metabolism in internodes become more active in *D. sinicus* from dormant to rapid growth stages. In short, the phytohormones were closely related to the rapid shoot growth of *D. sinicus*.

3.3. A key role of GAs in rapid growth of bamboos

GAs are phytohormones that play essential roles in internode elongation through enhancement of cell elongation and, in some cases, cell division (Nagai et al., 2020; Chen et al., 2022). To date, more than 100 kinds of GAs have been identified with GA1 and GA4 being the major bioactive ones in higher plants, and other GAs were non-bioactive and act as precursors of the bioactive forms (Hedden and Thomas, 2012). We thus identified the contents of various GAs separately. GA1, GA6, GA19, GA20, GA44 and GA53 were detected in GA1 pathway, but only GA4, GA34 and GA51 were detected in GA4 pathway (Fig. 1c). Furthermore, the level of GAs in GA1 pathway was higher than those in GA4 pathway, and the content of bioactive GA1 in the ST2 was significantly higher than other stages. This indicated that GA1 may be more important than GA4 in internode elongation during rapid shoot growth of *D. sinicus*.

To further investigate which phytohormone (IAA, GAs, CK, and ABA) was more closely related to the rapid shoot growth of *D. sinicus*, we tested correlations between growth rate and level of endogenous phytohormones in internodes (Fig. 1d). Growth rate showed significant correlations with ABA, GA19 and GA51. Furthermore, the total GAs, GA19, GA20 and GA51 were all significantly correlated with the ABA, as also observed between CK and the total GAs, GA19, GA53. Additionally, the correlation between IAA and ABA was significant. Our results confirmed that

of gibberellins in internodes during rapid growth in *D. sinicus*. Dashed boxes indicate not detected, colored boxes represent bioactive GAs (GA1 and GA4), and non-colored boxes are non-bioactive GAs. Data are mean ± s.d. (n = 3 shoots). Asterisks represent the content of shoots between different stages were varies significantly (Kruskal test). GA13ox: GA13-oxidase, GA20ox: GA 20-oxidase, GA3ox: GA 3-oxidase, GA2ox: GA 2-oxidase. (d) Correlations between growth rate and level of endogenous phytohormones of internodes in *D. sinicus* shoots. Each row and column correspond to growth rate or content of phytohormone, the circle is color-coded by correlation coefficient according to the color legend, circle sizes were negative correlated with p-value. Cells marked with asterisks represent traits were associated with each other significantly ($p < 0.05$). (e) The growth patterns of *P. edulis* shoots during fast growth of the 10th internodes. The growth patterns were similar in 9th, 10th, 11th, 12th internode, they all have a 'slow-fast-slow' growth pattern. When these internodes rapid growth were completed, the whole bamboo shoot is still growing rapidly. (f) The proportions of expression of GAs-related DEGs in total GAs-related genes in shoots of eight representative woody bamboos with different culm sizes. The blue represents the GAs-related DEGs expression proportions to the total expression measured for all GAs-related genes in the bamboo species, while the red represents GAs-related non-DEGs.

endogenous phytohormones and GAs in particular were closely correlated with internode elongation of *D. sinicus*.

Meanwhile, we calculated and compared the expression proportion of GAs-related DEGs in total GAs-related genes in representative woody bamboos with different culm sizes. The results showed that GAs-related DEGs were more highly expressed in the large-sized woody bamboos than the small-sized ones (Figs. 1a, f and S2). In addition, a significant positive correlation was found between the expression proportion of GA-related DEGs expression levels in total GAs-related genes and culm size of woody bamboos (Fig. 1f). These results further indicated that GAs was crucial in the rapid shoot growth and culm size regulation of woody bamboos.

3.4. Transcriptome analyses in woody and herbaceous bamboos

We further identified potential candidate genes of rapid shoot growth by WGCNA in *D. sinicus*, as well as in *P. edulis* (Fig. 1e) and *B. amplexicaulis*. To compare with an herbaceous bamboo (HB), we also performed transcriptome sequencing and analyses using data acquired from *Ra. guianensis* at different developmental stages (Fig. S1).

By estimating the module-trait relationships using the correlation between MEs and traits, modules that were significantly associated with growth rate, content of endogenous phytohormones in rapid growing shoots were identified in *D. sinicus*. The yellow module, comprising 1226 DEGs, exhibited a significant positive correlation with the growth rate, total GAs, GA6, GA44, GA51, and GA53 (Figs. 2a and S3). It was suggested that this module may regulate rapid shoot growth through gibberellins driving other factors. KEGG and GO enrichment analyses implicated many DEGs in this module involved in various pivotal biological processes during plant development (Figs. 2b and S4). In *P. edulis*, a total of 12,589 genes were identified as DEGs, which clustered into 16 modules ranging in size from 12 to 4581 genes by WGCNA (Fig. S5), we found that the blue module was significantly associated with growth rate. Furthermore, we discovered a positive correlation between the trend of gene expressions within this module and the growth rate (Fig. S6). Therefore, we speculated that these DEGs in blue module may positively regulate rapid shoot growth in *P. edulis*. Similar to *P. edulis*, a total of 10,174 genes were identified as DEGs, which clustered into 26 modules ranging in size from 24 to 2176 genes by WGCNA during rapid shoot growth in *B. amplexicaulis* (Figs. S7 and S8), the red module was most significantly associated with growth rate.

We conducted an in-depth comparison of the interaction network among key genes involved in key processes across three growth-related modules in three woody bamboos (Fig. 2c–e). In general, the number of key genes identified in the yellow module of *D. sinicus* and the blue module of *P. edulis* were similar, with 52 and 59, respectively. However, the red module from small-sized bamboo *B. amplexicaulis* displayed lesser number of key genes (38). Based on the above results, it seemed that the biosynthesis of GAs, IAA and lignin was essential during rapid shoot growth of three woody bamboos. And the metabolic processes shared by two large-sized woody bamboos (*D. sinicus* and *P. edulis*) also included transcription, cellulose and sugar metabolism. This suggested that the biosynthesis of cellulose and sugar were vital for rapid shoot growth of the woody bamboos. However, the metabolic processes in small-sized bamboo *B. amplexicaulis* was simpler than those in other two large-sized bamboos. We further analyzed the genes involved in metabolic pathways commonly found in three woody bamboos and thus identified the *SLRL1* gene shared by all three bamboos, which plays an important role in GAs signal transduction in model plants (Bao et al., 2020).

To identify which genes contributed to culms growth of herbaceous bamboos, we performed transcriptomic analysis of *Ra. guianensis* at three different developmental stages defined here (Fig. S1). In the initial stage (S1), the shoots were young with culm heights of approximating 2.5 cm. Subsequently, at S2, the shoots grew to 15 cm and continued to grow. By the final stage (S3), the culms were mature and elongation stopped while culms grew up to 29 cm. A total of 2692 DEGs were identified, KEGG and GO enrichment analysis showed that 111 DEGs were assigned to key biological processes (Fig. S9; Table S1). Nevertheless, no gene was enriched in lignin biosynthesis despite there were 34 genes involved in hormone pathways, making the largest proportion among the enriched DEGs (Fig. 2f). Remarkably, by comparing the transcriptomic results with woody bamboos, we found that *KAO* homologous to woody bamboos was also DEGs in the herbaceous *Ra. guianensis*. The results indicated that the *KAO* genes are essential for the biosynthesis of GAs in both herbaceous and woody bamboos, which is consistent with previous studies in other plants (Binenbaum et al., 2018). Interestingly, the *SLRL1* gene were not differentially expressed in culm development of herbaceous bamboo, it is thus inferred that *SLRL1* gene may be vital for rapid shoot growth and would have contributed to the origin of the innovative trait in woody bamboos.

Taken together, we identified two candidate genes associated with the rapid shoot growth of woody bamboos. The *KAO* and *SLRL1* might be responsible for GAs biosynthesis and signal transduction in bamboos, respectively.

3.5. Expression divergence of GAs-Related genes among subgenomes

We further identified the homoeologs of GA-related genes in 11 bamboos and investigated their expression patterns in young shoots. As expected, the largest number of GAs-related genes were identified in three hexaploid PWBs, followed by six tetraploid woody bamboos, and the least in two herbaceous bamboos (Fig. 3e; Table S2). More gene copies may provide a genetic basis for complex biological processes in woody bamboos compared with herbaceous bamboos.

We next traced homoeolog expression patterns for GAs-related DEGs in *D. sinicus*, *B. amplexicaulis* and *P. edulis* during rapid shoot growth. In *D. sinicus*, 30 DEGs were identified (Fig. 3a). These genes encode major protein families known to be involved in GAs biosynthesis and signal transduction, and their expression levels varied substantially at different developmental stages (ST1, ST2, ST3 and ST4) and among the subgenomes A, B and C. Overall, we found the total gene expression of DEGs was dominated by the subgenomes A and C, except for ent-kaurene synthase (*OsKS1*). In *P. edulis*, we detected 22 GAs-related DEGs (Fig. 3b). They varied substantially both in the number and expression levels between subgenomes C and D. There were 15 copies of DEGs on subgenome C, in sharp contrast to only seven copies on subgenome D. Furthermore, the expression levels of most DEGs on subgenome C were higher than homoeolog DEGs on D, indicating the dominance of subgenome C during rapid shoot growth. In *B. amplexicaulis*, we identified 16 copies of GAs-related DEGs (Fig. 3c). Unlike the observations in *D. sinicus*, the expression levels of DEGs on the subgenome B were higher in *B. amplexicaulis*, and it could be called the subgenome B dominance here.

Enrichment analysis of GAs-related DEGs expression to subgenome showed that in two hexaploid PWBs, DEGs with high expression levels were significantly enriched on subgenome A in *D. sinicus* while instead on subgenome B in *B. amplexicaulis*. In tetraploid TWB *P. edulis*, GAs-related DEGs on subgenome C was preferentially expressed (Table S3). These results reflecting the

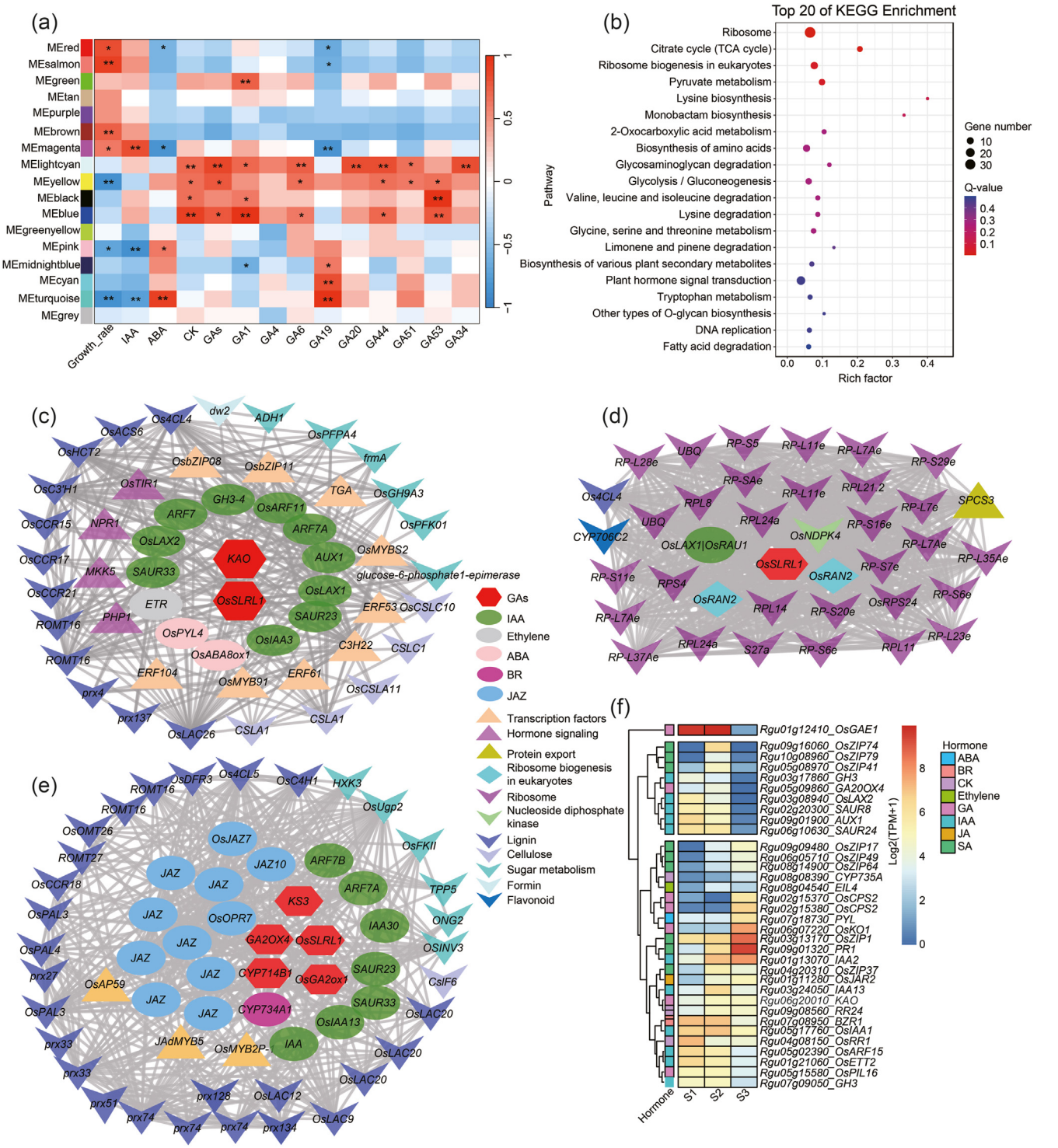

Fig. 2. Key genes associated with the rapid growth in woody bamboos and herbaceous bamboo. The abbreviations were defined in Table S6. (a) Correlation between co-expressed modules and sampling traits in *Dendrocalamus sinicus* during rapid growth by WGCNA. Each row corresponds to a module, column to a trait. The table is color-coded by correlation according to the color legend. Growth_rate: daily increments of 10th internode of *D. sinicus* shoots, GAs: content of total gibberellins, CK: content of cytokinin, IAA: content of auxin, ABA: content of abscisic acid. GA1, GA4, GA6, GA19, GA20, GA44, GA51, GA53 and GA34 were similar to above. Cells marked with asterisks represent trait was associated with module each other significantly ($p < 0.05$). The yellow module was significantly positively correlated with Growth_rate, CK, GAs, GA1, GA6, GA44, GA51, GA53, indicated that this module may regulate rapid growth by various gibberellins. (b) KEGG pathway annotations of DEGs in yellow module in *D. sinicus* transcriptome during rapid shoot growth. The *y*-axis was the KEGG pathway name, the *x*-axis represents the rich factor. The size of each point corresponding to the gene number involved in the KEGG pathway, the color of each point according to the color legend of -log10 (Q value). (c–e) Co-expression network of the hub genes from growth-related module in *D. sinicus*, *Phyllostachys edulis* and *Bonia amplexicaulis* shoots transcriptome during rapid growth. (c) *D. sinicus*, (d) *B. amplexicaulis*, (e) *P. edulis*. (f) Expression patterns of phytohormone-related DEGs in *Raddia guianensis* culms. The heatmap was generated from their transcripts per kilobase million mapped reads (TPM) from RNA-seq analysis. Color bar is the scale for the expression levels of each gene. The colored rectangles on the left show the phytohormone to which the gene belongs. The right column shows the corresponding gene IDs and gene names, and samples are labelled below the heatmap.

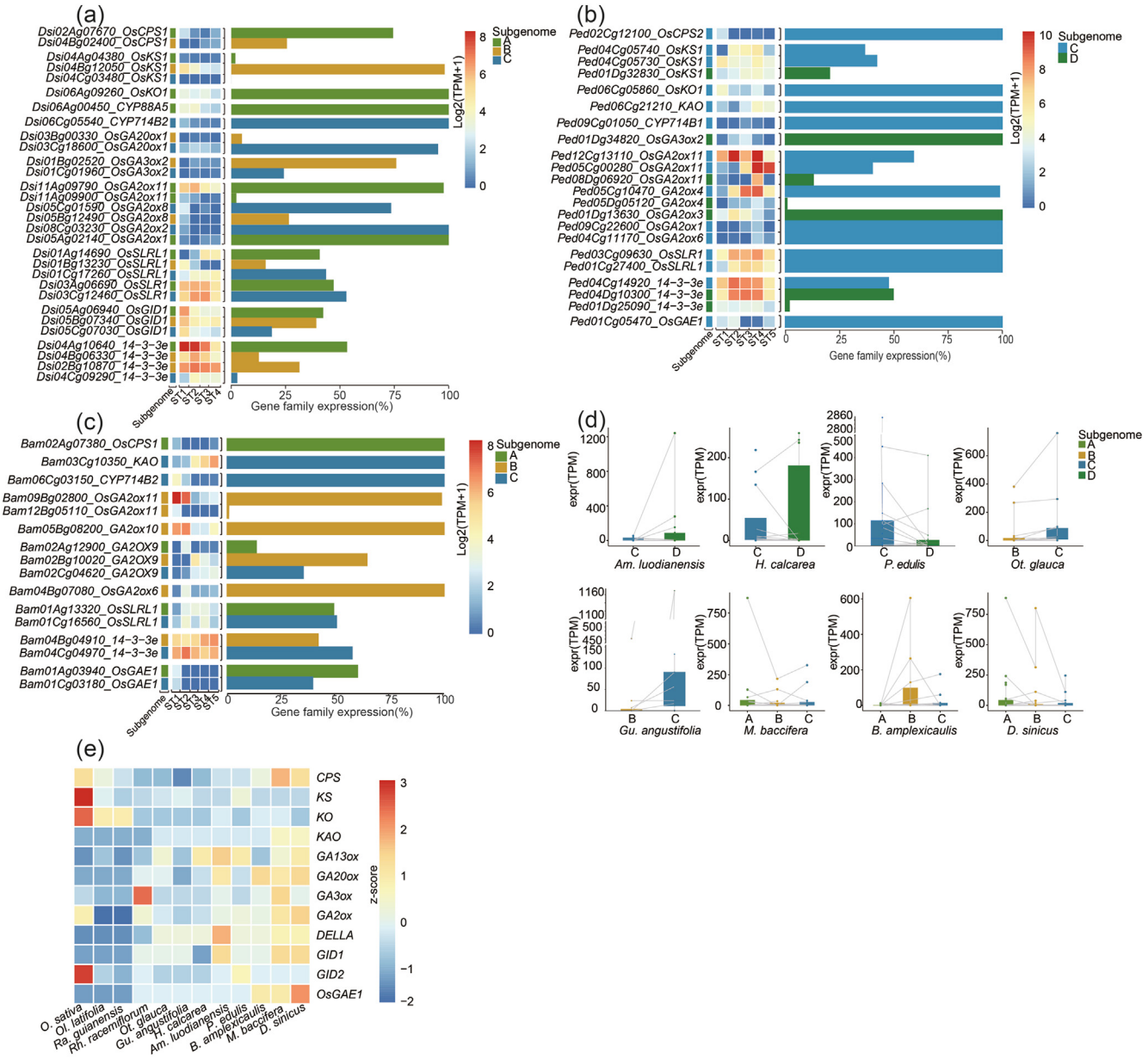

Fig. 3. GAs-related genes in bamboos and their expression patterns in woody bamboos. (a–c) Expression patterns of GAs-related genes in *Dendrocalamus sinicus*, *Phyllostachys edulis* and *Bonia amplexicaulis* shoots during rapid shoot growth. The left column shows the corresponding gene IDs and gene names. The heatmap was generated from their transcripts per kilobase million mapped reads (TPM) from RNA-seq analysis. Color bar is the scale for the expression levels of each gene. The colored rectangles on the left of heatmaps show the subgenomes to which the gene belongs, and samples are labelled below the heatmap. Bar charts visualize the expression contribution of individual GAs-related DEGs at different subgenomes to the total expression measured for all homoeolog DEGs. (a) *D. sinicus*, (b) *P. edulis*, (c) *B. amplexicaulis*, (d) Expression patterns of homoeolog GAs-related DEGs in eight woody bamboos shoots. Bar charts visualize the mean expression level of genes which were homoeologous to GAs-related DEGs during rapid growth in *D. sinicus*, *P. edulis* and *B. amplexicaulis* shoots. Dots represent expression level of a DEG and dots linked by lines represent homoeolog copies at different subgenomes in individual bamboo species. (e) Gene copies of GAs-related gene families in 11 representative bamboos and rice.

greater contribution of individual gene copies on subgenomes A and C than B and D to rapid shoot growth of *D. sinicus* and *P. edulis*, respectively. However, in small-sized *B. amplexicaulis* (Fig. 1a), GAs-related genes on subgenome B may be dominant over both subgenomes A and C.

We examined the expression patterns of homoeolog GAs-related DEGs among different subgenomes in individual bamboo species using young shoots of eight woody bamboos of different culm size. In TWBs, the highly expressed homoeolog copies were on subgenome C in *P. edulis*, which is in accordance with expression pattern during rapid shoot growth, while the highly expressed homoeolog copies in *Am. luodianensis* and *H. calcarea* were on subgenome D. In PWBs, the expression patterns in *B. amplexicaulis* and *D. sinicus* were consistent with observations during rapid shoot growth, and gene copies on subgenome A were dominant over both subgenomes B and C in *M. baccifera* shoots. The expression levels of gene copies on subgenome C were higher than subgenome B in two tetraploid NWBs (Fig. 3d; Table S4).

Enrichment analysis of GAs-related DEGs expression with respect to subgenome in young shoots suggested that these results were significant and solid (Table S3). In all, GAs-related genes on the subgenomes A and C were predominant over B or D in large-sized species, whereas the trend is converse in small-sized woody bamboos.

3.6. Subgenome-specific gene structure

To explore whether the gene structure had an effect on the transcriptional expression of the genes from different subgenomes, we identified all *KAO* and *SLRL1* gene members in 11 bamboos.

A total 20 *KAO* genes were identified in 11 bamboos genomes using rice *KAO* sequences as queries, with one gene on each subgenome except for subgenome C of *Am. luodianensis*, subgenome B of *Rh. racemiflorum* and *B. amplexicaulis* (Figs. 5a and S10a). *KAO* genes were located in a conserved syntenic region of chr6 between rice and bamboo subgenomes. However, an inter-chromosomal translocation of a segment within *KAO* located occurred between chrs 3 and 6 of subgenome C in six NWB and PWB species (Fig. S10a). To explore the evolutionary relationship of the *KAO* genes in bamboos, a phylogenetic tree was constructed and 20 copies were clearly grouped based on subgenomes A, B, C, D, and H. Notably, the total expression of *KAO* in shoots was all dominated by the C copy in woody bamboos, except for *D. sinicus* (Fig. 4a). And the expression of genes on subgenomes B or D was suppressed in all woody bamboos.

As UTR-exon-intron distribution patterns, motifs and domains of *KAO* genes were very conserved (Fig. 5), the sequences of their DNA and protein are too similar to have effect on homoeolog expression divergence between subgenomes. However, UTR was variable among these 20 genes. In detail, almost all of genes on subgenomes A, C and H contained UTRs, but all genes on the subgenomes B and D did not have (Fig. 5). Remarkably, this pattern was consistent with the expression trend of the *KAO* genes in woody bamboo shoots. These results demonstrate that differences of UTRs may result in homoeolog expression divergence among subgenomes, which is the basis for the functional divergence of genes in bamboos, providing a subgenome-specific gene structure for studying the unique traits in woody bamboos.

A total 22 *SLRL1* genes were identified in 11 bamboos genomes, with one gene on each subgenome except for subgenome B of *Rh. racemiflorum* (Figs. 6a and S10b). The *SLRL1* genes were located in a well-conserved syntenic region of chr1 between rice and bamboo subgenomes, and the 22 gene copies were also clustered based on subgenomes. Interestingly, the total expression of *SLRL1* in shoots was generally dominated by A and C copies in PWBs, and all dominated by C in NWBs and TWBs. The expression of genes on subgenomes B and D was suppressed in all woody bamboos (Fig. 4b).

Investigation of gene structure and proteins uncovered that UTR-exon-intron distribution patterns, motifs and domains of *SLRL1* genes were conserved among subgenomes (Figs. S11 and S12). We thus further analyzed the promoter region of these genes and identified numerous CREs distributed in the promoter regions, which could be classified into nine fundamental functional categories (Fig. 6d). Furthermore, the number of these CREs also varies among gene copies, particularly for the core promoter, which represents a high proportion in CREs in most *SLRL1* genes. In individual species, the numbers of core promoter of genes from different subgenomes were positively correlated with the transcriptional expression level (Fig. 6d and Table S5). For example, genes on subgenomes A and C have more core promoters while the gene expression is higher in *M. baccifera*. In *P. edulis* and *Am. luodianensis*, the number of the core promoters on subgenome D was low, so as to the gene expression. We also found that core promoters were more distributed in non-conserved regions among different genes across rice and bamboos. These results implied that the differences (in number and position) of the core promoter may contribute to the gene expression, resulting in homoeolog expression divergence among subgenomes.

4. Discussion

Bamboos have experienced multiple rounds of intricate hybridizations and polyploidizations, resulting in a range of ploidy levels and subgenome compositions (Ma et al., 2024). The significance of subgenome dominance in plants has been increasingly recognized and believed to have adaptive plasticity, thereby shaping the evolution and diversifications of plants (Alger and Edger, 2020). This plasticity has facilitated the domestication and

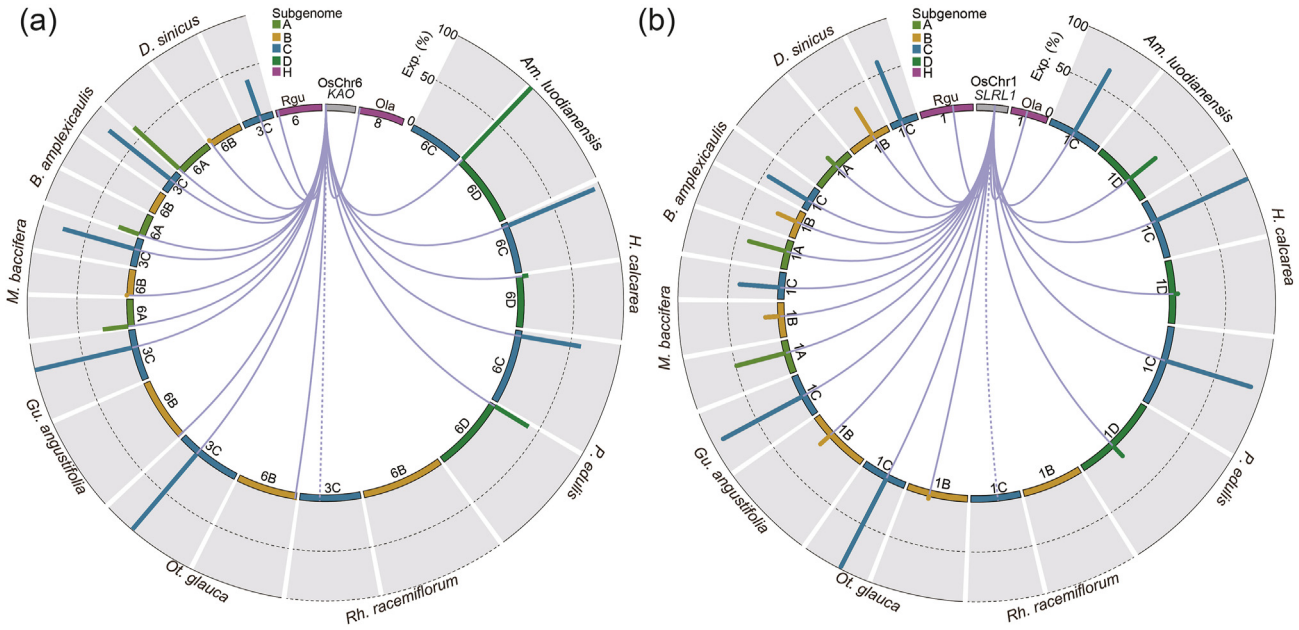

Fig. 4. The expression patterns of *KAO* and *SLRL1* in woody bamboo young shoots. The abbreviations were defined in Table S6. The inner colored track indicates each chromosome belong to different subgenomes. The inner connecting purple lines represent homoeologs connections among rice and bamboos, dotted purple line linking rice and *Rhipidocladum racemiflorum* indicated without transcriptomic data in *Rh. racemiflorum* shoots. The bars joining with purple lines was the expression proportion of individual gene to the total expression of all homoeolog copies in individual bamboo species. (a) *KAO*. (b) *SLRL1*.

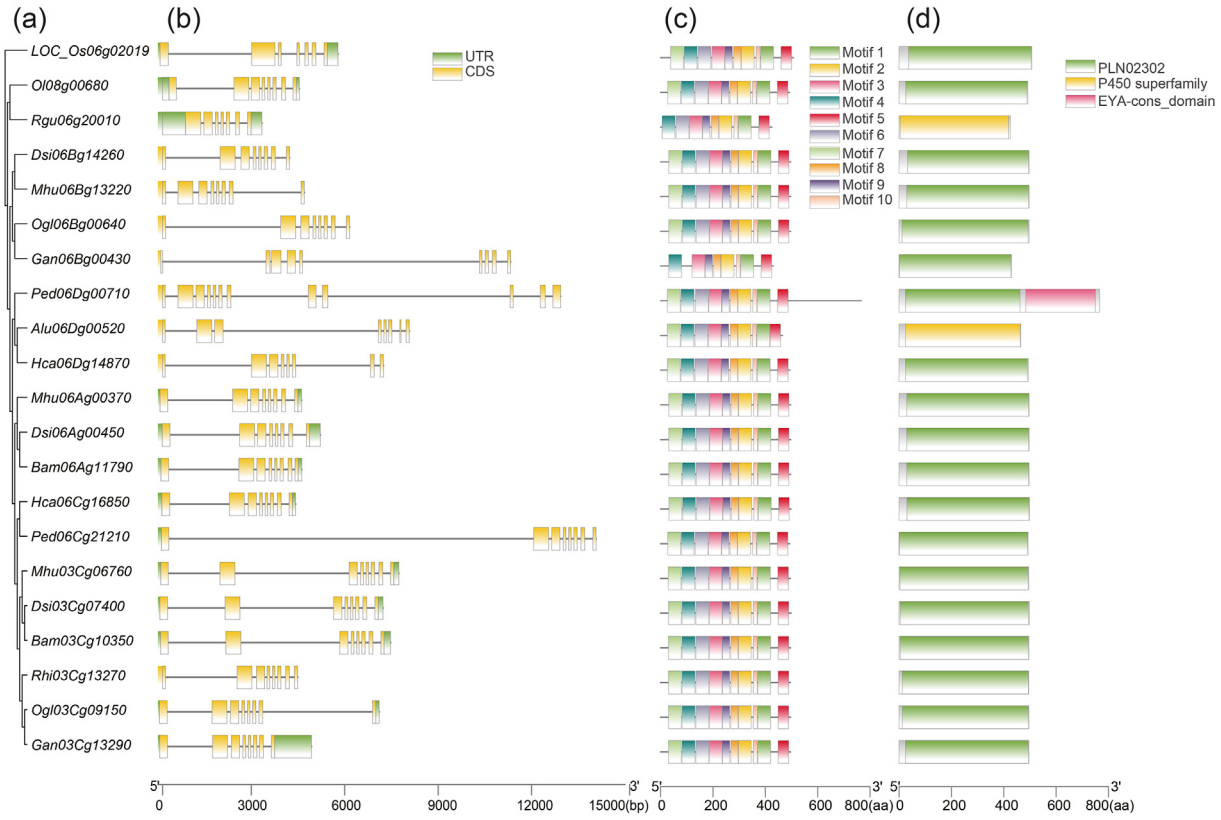

Fig. 5. The gene structure comparison of *KAO* gene across rice and 11 bamboos. (a) Phylogenetic tree of the *KAO* gene copies. (b) UTR-exon-intron distribution patterns of *KAO* gene copies. Exons and UTRs, introns are represented by colored boxes and black lines, respectively. (c) Conserved motifs of *KAO* gene copies identified by MEME analysis. Each motif was represented with different color. (d) Domains in *KAO* gene copies.

adaptation of several major domesticated crops, for example, hexaploid bread wheat (*Triticum aestivum*) (Pfeifer et al., 2014; Wang et al., 2016; Ramirez-Gonzalez et al., 2018), allotetraploid cotton (*Go. hirsutum*) (Wang et al., 2017; Bao et al., 2019; Dong et al., 2022; You et al., 2023), and the resynthesized allotetraploid *Brassica napus* (Bird et al., 2021). Our previous work on genome assemblies across 11 bamboo species revealed an empirical case that large scale species diversification of woody bamboos could be induced by dynamic subgenome dominance (Ma et al., 2024).

The rapid shoot growth of woody bamboos was a key innovative and distinguishing trait within the grass family. Recent studies found that monthly precipitation may regulate the internode size by affecting the primary thickening growth of *P. edulis* (Zhang et al., 2024), and the 'PeSAPK4-PeMYB99-*PeTIP4-3*' regulatory model might be involved in water transport during its shoot growth (Zhu et al., 2024). Genome-wide analysis in this bamboo identified the *KNOX* (KNOTTED1-like homeobox), *GRF* (Growth-regulating factors) and aspartic protease gene families in promoting the rapid shoot growth (Jiao et al., 2024; Wang et al., 2021c; Zhou et al., 2023). And analyses of 77 woody bamboos found that the heritable legacy dominates development of culms functional traits (Liu et al., 2024b). Our study suggests that the genetic basis of adaptation and subsequent diversification in bamboos would further benefit from in-depth investigation using multiple bamboo species with different ploidal levels and culm sizes.

4.1. Importance of GAs in rapid growth and culm size of bamboos

By summarizing a large number of previous studies on the growth patterns of bamboo culms, we found that woody bamboos share a similar growth pattern, and large bamboos, particularly the tetraploid *P. edulis*, usually grow faster than small ones during the rapid growth stages (Wang et al., 2012a; Huang et al., 2016; Tan and Wu, 2018; Xu, 2018; Chen et al., 2022; Niu et al., 2022).

Quantification of the phytohormones in *D. sinicus* showed that the changes in content of GAs and CK had similar trends with *P. edulis* during the rapid growth with the highest level found in the ST2. The ST2 was the initiation period of rapid internode elongation, which might undergo vigorous cell division and metabolisms for synthesis of materials synchronously (Niu et al., 2022; Ma et al., 2024). Furthermore, the level of GAs was higher in the internode cell division zone with a relatively higher cell division intensity, closely correlated with the CK content in the internode of *P. edulis* during the rapid growth (Chen et al., 2022). So, we hypothesized that GAs and CK also cooperated with each other to regulate cell division and metabolisms of internode in *D. sinicus*.

Notably, GAs was significantly correlated with other important phytohormones (Fig. 1d). One possible explanation for these observations might be that GAs was the hub phytohormone that can interact with other phytohormones to regulate rapid shoot growth in *D. sinicus*, as observed in *P. edulis* (Tao et al., 2018; Chen et al., 2022). The proportion of GAs-related DEGs expression levels in representative woody bamboos with different culm sizes also indicated that GAs was crucial in the rapid shoot growth and size regulation of bamboos (Fig. 1a and f). Indeed, GAs can antagonistic crosstalk with ABA by distinct TF regulators and the direct molecular interaction between core signaling components, thereby regulating plant growth and development (Liu and Hou, 2018). GAs can also crosstalk with other phytohormones through DELLA protein, which together regulate various developmental processes in plants (Daviere and Achard, 2016; Bao et al., 2020).

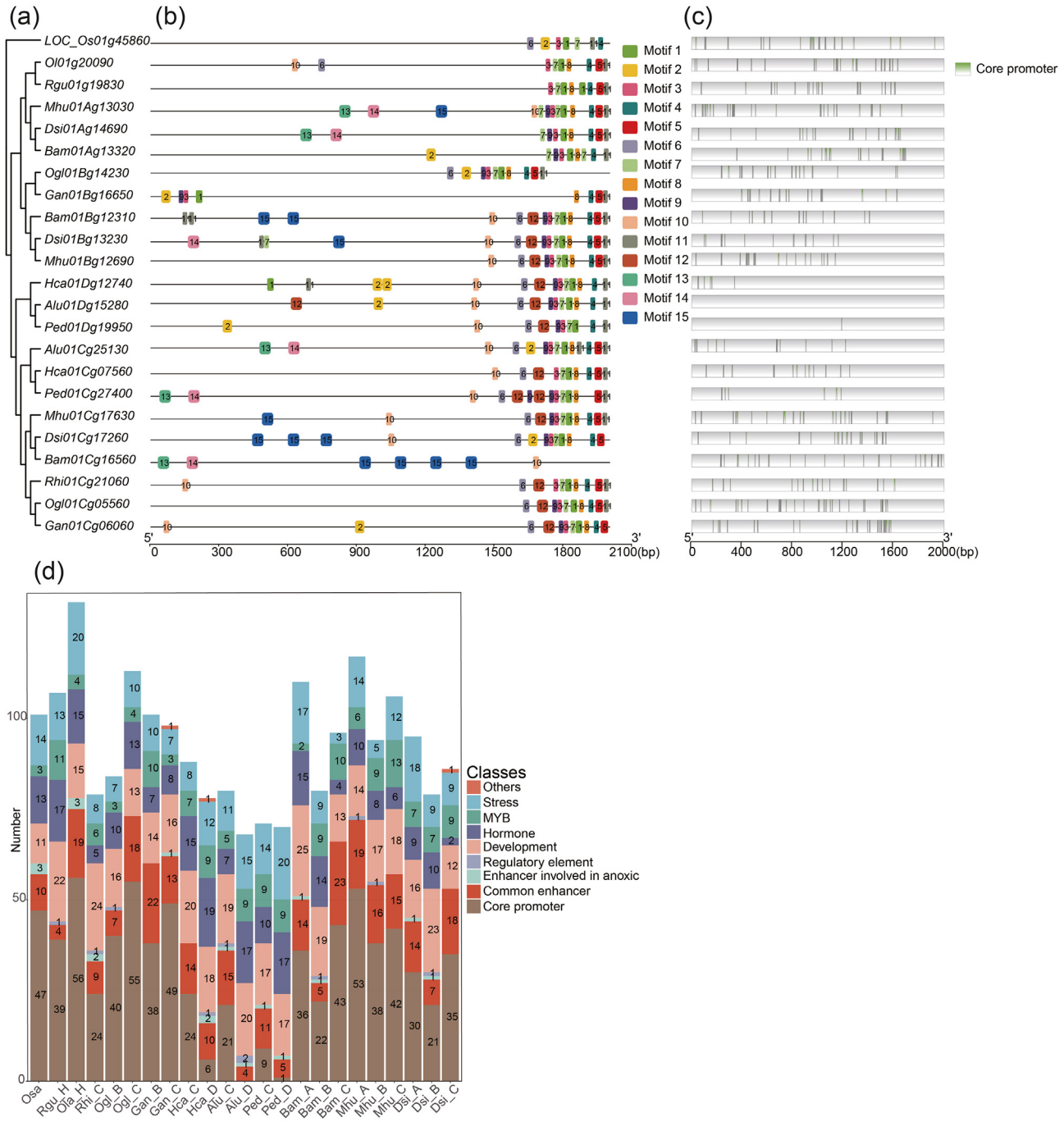

Fig. 6. The cis-regulatory elements analyses of *SLRL1* gene across rice and 11 bamboos. (a) Phylogenetic tree of the *SLRL1* gene copies. (b) Conserved motifs of 2000 bp upstream at *SLRL1* gene copies identified by MEME analysis. Legend depicting the color of individual motif. (c) The core promoter distribution of 2000 bp upstream at *SLRL1* gene copies. (d) The number summary of cis-regulatory elements (CREs) in the promoter region of *SLRL1* gene copies at different subgenomes.

Analyses of various kinds of GAs found that there were two pathways to synthesize bioactive GAs in *D. sinicus* (GA1 and GA4 pathway) (Fig. 1c), which is consistent with previous study in *P. edulis* (Gamuyao et al., 2017). Meanwhile, some non-bioactive GAs would also be generated (Hedden and Thomas, 2012). By comparing various GAs contents on these two pathways, we found that the type of GAs that actually regulates rapid shoot growth in *D. sinicus* might be GA1. On the contrary, previous studies suggested that GA4 may be more important in *P. edulis* (Chen et al., 2022).

By integrating transcriptome analysis results of rapid shoot growth from three woody bamboos (*D. sinicus*, *P. edulis* and *B. amplexicaulis*), we identified a candidate gene *SLRL1* that were associated with the rapid growth in all of them. *SLRL1* gene encodes a DELLA protein that belongs to a subfamily of the GRAS protein superfamily and that functions as a repressor of GA signaling (Fukao and Bailey-Serres, 2008), DELLA proteins were the master negative regulators of GA signaling, and can integrate multiple hormones signaling pathways, such as cross-talking of GAs with IAA, CK, BR, ABA and JA, and thus are involved in

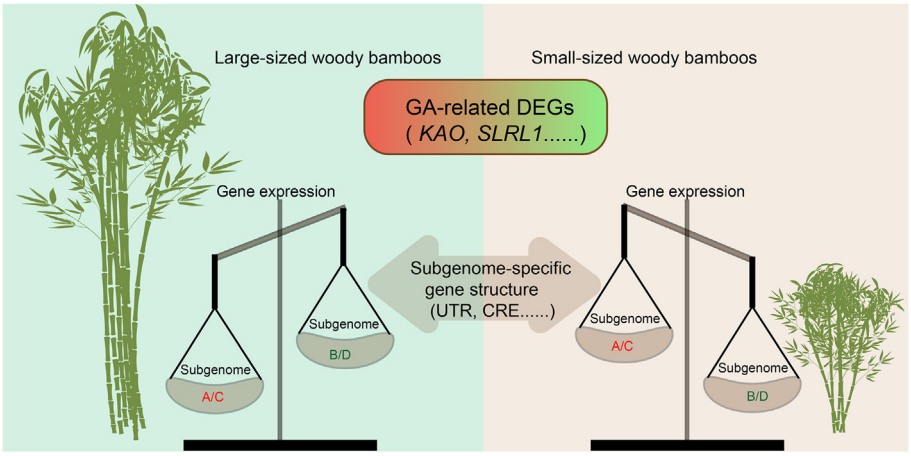

Fig. 7. Proposed model for subgenome asymmetry of gibberellins-related genes regulates rapid shoot growth in woody bamboos. The expression levels of GA-related DEGs may be positively regulated culm size of woody bamboos. Furthermore, the key GA-related genes on dominant subgenomes A and C demonstrating expression dominance in the large-sized woody bamboos while the generally submissive subgenomes B and D dominating in the small-sized species. This subgenome asymmetry may be caused by subgenome-specific gene structure.

multiple biological processes in plants (Itoh et al., 2005; Claeys et al., 2014; Daviere and Achard, 2016; Bao et al., 2020). Three DELLA genes, *SLR1*, *SLRL1* and *SLRL2*, were identified in rice (Itoh et al., 2005; Thomas et al., 2016). Interestingly, *SLRL1* and *SLRL2* lack the N-terminal DELLA and TVHYNP domains, and the existence of SLRL-type GRAS proteins appears to be restricted to monocots (Hauvermale et al., 2012). The *SLRL1* was found to be expressed after submergence in deepwater rice, but not for *SLR1* (Fukao and Bailey-Serres, 2008) and it is inferred that *SLRL1* may be more sensitive to environmental stimulus than *SLR1* (Minami et al., 2018). Consistent with the findings in rice, we identified *SLR1*, *SLRL1* and *SLRL2* genes in the genomes of 11 representative bamboo species (Fig. S12). Through transcriptome data from rapid growing shoots of three woody bamboos, we further found that the *SLRL1* gene was shared by all three woody bamboos during rapid growth, but not differentially expressed in culm development of herbaceous bamboos. The *SLRL1* gene was thus more important for rapid growth and may contribute to the origin of the innovative trait in woody bamboos.

In *D. sinicus* and *P. edulis*, *SLRL1* gene might interact with IAA, BR, JAZ, ABA and ethylene by transcription factor, then regulating the lignin, cellulose, sugar and formin metabolism. However, in *B. amplexicaulis*, the crosstalk of phytohormones with GAs via the *SLRL1* gene was reduced, with only IAA identified. Simultaneously, the biosynthesis processes were reduced, only one lignin-associated gene and a flavonoid-associated gene were identified (Fig. 2d). The lignin content in the mature culms of *B. amplexicaulis* was significantly lower than that in *D. sinicus* and *P. edulis* (Ma et al., 2024), and its size was significantly smaller than *D. sinicus* and *P. edulis*, too (Fig. 1a). It indicates that the larger the bamboo, the more complex the biological processes were involved during rapid growth.

By comparing the transcriptomic results of the largest woody bamboo (*D. sinicus*) and a small herbaceous bamboo (*Ra. guianensis*), we identified the *KAO* gene essential for the biosynthesis of GAs in both species. *KAO* genes can regulate plant growth, in *Ar. thaliana*, the level of GAs and plant size in kao mutants were substantially reduced compared with the wild-type (Regnault et al., 2014). Lesions in *KAO* have been shown to be the cause of severe dwarfism in several model species, including rice (*d35* mutant) (Sakamoto et al., 2004), maize (*dwarf3*) (Helliwell et al., 2001), pea (*na*) (Davidson et al., 2003) and sunflower (*dwarf2*) (Fambrini et al., 2011).

4.2. Importance of dominant subgenome in rapid growth of woody bamboos

In our previous work, it was revealed that subgenome dominance could be established after polyploidization and inherited by their descendants, thereby contributing to the evolution of unique traits across woody bamboos (Ma et al., 2024). In this study, by analyzing the transcriptome of young shoots from eight representative woody bamboos, we investigated homoeolog expression patterns for GAs-related DEGs during rapid shoot growth in the woody bamboos. Generally, GAs-related genes on subgenomes A and C were predominant over subgenomes B and D in large-sized woody bamboos, conversely in small-sized ones. It was revealed that subgenomes C and A were dominant subgenomes in woody bamboos (Ma et al., 2024). Our findings indicate that dominant subgenomes positively regulates rapid shoot growth and the submissive subgenome also play important roles in regulation of culm size in woody bamboos. Similarly, submissive subgenomes were crucial in adaptation of radiated polyploid lineages in *Nepenthes* (Saul et al., 2023). In fact, the phenomenon that the patterns of transcriptome asymmetry were associated with their morphological traits were observed in tetraploid wheats (Wang et al., 2016) and cotton (You et al., 2023). The subgenomic or transcriptomic functional asymmetry has been shown to play critical roles in allopolyploid speciation and adaptation to the environments (Wang et al., 2021b). Specially, the patterns and mechanisms of homeolog biased expression pattern related to rapid growth and culm sizes in woody bamboos are not yet fully understood, calling for further investigation.

4.3. Genetic basis driving species diversification of woody bamboos

By analyzing the homoeolog expression pattern, gene structure, and conserved non-coding regulatory of key genes in 11 bamboos, we found that the homoeolog expression divergence among subgenomes in *KAO* genes may be closely related to UTRs. UTRs can play a crucial role in post-transcriptional control by regulating various mRNA properties like stability, transport, translation efficiency and functioning and subcellular localization of the translated proteins (Srivastava et al., 2018). The variation in UTRs of *KAO* genes on different subgenomes in woody bamboos may regulate the expression level by affecting properties of mRNAs.

The homoeolog expression divergence among subgenomes of *SLRL1* genes may be related to the core promoter in 2000 bp upstream of the gene body. Within the same bamboo species, the number of core promoters was positively correlated with the transcriptional expression level. A recent study in cotton suggests that diversity in the transcriptional regulation region for homoeolog genes may result in homoeolog expression divergence (You et al., 2023). Transcriptional regulation is mainly mediated by transcription factors interacting with cis-regulatory elements like core promoters (Shafee and Lowe, 2017; Bottani et al., 2018).

Our results from homoeolog expression patterns of *KAO* and *SLRL1* genes revealed that gene copies which exhibited subgenome-biased expression in woody bamboos tend to have subgenome-specific cis-regulatory sequences, similar to the results in cotton (Wang et al., 2017; You et al., 2023). This phenomenon will help us to understand the molecular basis for species diversification in woody bamboos following polyploidizations.

5. Conclusion

In this study, we found GAs may regulate rapid shoot growth and thus the mature culm size of woody bamboos, and GA1 could be the main type of bioactive in the largest bamboo *D. sinicus*, a representative of the PWB clade (Liu et al., 2024a). Meanwhile, we also identified both *KAO* and *SLRL1* involved in the GAs biosynthesis as important candidate genes for regulating the rapid shoot growth. By comparing the genomic and transcriptomic data of 11 representative bamboos, we found that expression level of GAs-related genes at subgenomes A and C were predominant over subgenomes B and D in rapid shoot growth of large-sized woody bamboos. This subgenome asymmetry in woody bamboos may be closely related to their adaptation and species diversification after multiple rounds of hybridizations and polyploidizations. Furthermore, we found intriguing correlations between subgenome asymmetry and subgenome-specific gene structure, e.g., UTRs and core promoters (Fig. 7). These findings provide new insights into the genetic mechanism of innovative traits regulation in polyploid woody bamboos and the subsequent species diversification.

CRediT authorship contribution statement

Ling Mao: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Cen Guo:** Software, Methodology, Investigation, Data curation. **Liang-Zhong Niu:** Methodology, Investigation, Formal analysis. **Yu-Jiao Wang:** Software, Methodology, Investigation. **Guihua Jin:** Methodology, Investigation. **Yi-Zhou Yang:** Software, Investigation. **Ke-Cheng Qian:** Software, Investigation. **Yang Yang:** Investigation. **Xuemei Zhang:** Investigation. **Peng-Fei Ma:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. **De-Zhu Li:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Zhen-Hua Guo:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Data availability

RNA-seq data are available at NCBI (accession: PRJNA948693).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pld.2024.10.004.

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