

Epigenomic reprogramming underlies internodal developmental heterogeneity in rapidly elongating bamboo shoots

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Abstract

Shoots of Ma bamboo (*Dendrocalamus latiflorus*) are widely valued for their high yield, extended harvest period, and nutritional richness. Despite their economic and ecological importance, the epigenetic mechanisms governing shoot development remain largely unexplored. Here, we integrated cytological, transcriptomic, proteomic, and multi-epigenomic analyses to characterize the developmental gradient from the basal mature region to the apical proliferative zone. Cytological observation revealed a transition from structurally reinforced cells at the basal internode to actively dividing cells at the apical internode. Genome-wide bisulfite sequencing showed a progressive increase in CG methylation and a decrease in CHG methylation within gene bodies, accompanied by promoter-enriched CHH methylation that was positively correlated with transcriptional activation. Chromatin profiling demonstrated elevated H3K36me3 active marks and enhanced chromatin accessibility at the apical internode. In contrast, N⁶-methyladenosine (m⁶A) levels declined from the basal internode to the apical internode, coinciding with the activation of growth-related genes. Using nanopore-based sequencing, we further found that poly(A) tail lengths (PALs) were positively associated with m⁶A accumulation and gene body CG methylation but negatively correlated with H3K27me3 and H3K36me3 levels. Together, this study establishes an integrated epigenetic framework in which DNA methylation, histone modifications, m⁶A, and PAL dynamics collectively fine-tune transcription and translation during bamboo shoot elongation. Our study provides a multi-epigenomic model for *D. latiflorus* development and broadens our understanding of epigenetic coordination in a monocot, thereby offering valuable insights for future genetic improvement.

Introduction

Bamboo plants belong to the subfamily Bambusoideae of the Poaceae family, comprising approximately 130 genera and over 1,700 species. Based on the morphology of underground stems, bamboos can be classified into 3 categories: clumping bamboos, running bamboos, and mixed-type bamboos. Running bamboos, such as Moso bamboo (*Phyllostachys edulis*) (Zhao et al. 2018), and clumping bamboos, such as Ma bamboo (*D. latiflorus*) (Zheng et al. 2022), are important forestry resources due to their rapid growth, high mechanical strength, and substantial economic value.

With the advancement of high-throughput sequencing technologies, chromosome-level genomes of many bamboo species have been gradually released. The draft genome of *P. edulis*, a representative running bamboo, was published with annotations of genes involved in cellulose and lignin biosynthesis in bamboo shoots (Peng et al. 2013). Subsequently, using Hi-C technology, the genome of *P. edulis* was assembled to the chromosome level (Zhao et al. 2018), paving the way for further pan-genome (Hou et al. 2024) and resequencing studies (Zhao et al. 2021; Liu et al. 2024). Leveraging these genomic references, researchers have explored the role of DNA methylation and small RNAs in the epigenetic regulation of the transition from

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vegetative to reproductive growth (Zhang et al. 2021; Yu et al. 2025). During its rapid growth phase, *P. edulis* can exhibit simultaneous elongation in up to 40 internodes. Its internode growth is modular, involving distinct zones of cell division, cell elongation, and secondary cell wall thickening (Chen et al. 2022). Studies on different internode regions have revealed the involvement of microRNAs in regulating rapid growth (Wang et al. 2021). Additionally, dynamic RNA methylation modifications in internodes of bamboo shoots at varying heights have been demonstrated (Li et al. 2023).

Beyond *P. edulis*, the genome of 4 additional bamboo species have been sequenced (Guo et al. 2019), representing major branches of Bambusoideae—including the herbaceous bamboos *Olyra latifolia* and *Raddia guianensis*, as well as the woody bamboos *Guadua angustifolia* and *Bonia amplexicaulis*. More recently, chromosome-level genomes for 11 bamboo species were assembled (Liu et al. 2024; Ma et al. 2024) and a comprehensive bamboo research database was established (Liu et al. 2024), providing valuable molecular resources for bamboo research.

The genome assembly of the hexaploid clumping bamboo *D. brandisii* revealed that the expression levels of key enzyme genes in lignin biosynthesis pathway (such as 4CL, CCR, and F5H) are substantial lower than those in *P. edulis* (Jiang et al. 2024). In contrast, genes associated with shoot sweetness regulation are markedly upregulated in *D. brandisii*, explaining its tender, fresh, and sweet taste (Jiang et al. 2024). In addition to *D. brandisii*, *D. latiflorus* is another economically and ecologically important bamboo resource in China. The genome assembly of hexaploid *D. latiflorus*, a representative clumping bamboo, has provided key insights into the clues underlying bamboo shoot abortion (Zheng et al. 2022). Although DNA methylation (Niu et al. 2022; Li et al. 2023; Xi et al. 2023; Zou et al. 2024; Yu et al. 2025) and RNA methylation (Li et al. 2023; Liufu et al. 2023; Wang et al. 2025a, 2025b) have been reported to play roles in the regulation of *P. edulis* development, the cytological and epigenetic mechanisms underlying shoot development in *D. latiflorus* remain largely unexplored.

Notably, *P. edulis* and *D. latiflorus* show distinct shoot emergence patterns: shoots of *D. latiflorus* develop from buds at the base of the culm, whereas those of *P. edulis* arise from lateral buds on underground rhizomes. Therefore, in this study, we conducted cytological observations of serial internode sections from *D. latiflorus* shoots. Representative samples from the second (S2), fourth (S4), and sixth (S6) internodes were selected for multi-omics analysis, including BS-seq, ChIP-seq of 2 histone modifications (H3K27me3 and H3K36me3), ATAC-seq for chromatin accessibility, Nanopore direct RNA sequencing (DRS), and proteome sequencing. Through comprehensive analysis of gene expression, protein translation, histone modifications, and chromatin openness across different internodes, we identified a set of epigenetically regulated genes. This finding provides a theoretical foundation for revealing the epigenetic regulation of shoots growth in *D. latiflorus*.

Materials and methods

Samples from 3 different internodes

Experimental materials were collected from shoots grown under uniform conditions in Zhangzhou City, Fujian Province (E117°27'36", N24°39'49"). Bamboo shoots approximately 1.6 m tall were harvested in summer shortly after emerging above the soil surface. To represent different developmental stages, the second (S2), fourth (S4), and sixth

(S6) internodes were sampled. For each internode, a 1-cm segment from the basal region of each internode was excised (Fig. 1a), with 3 biological replicates collected per stage. We immediately froze the samples in liquid nitrogen and stored at -80°C for high-throughput library construction and sequencing. The remaining tissues were washed with ultrapure water, dried with filter paper, and a 1-cm block from the center of the ring was placed in FAA fixative for anatomical observations.

DNA extraction, bisulfite sequencing, and DNA methylation analysis

Filtered clean data (FASTQ format) was aligned to the *D. latiflorus* genome (Zheng et al. 2022), with the genomic sequence and annotation files downloaded from <https://doi.org/10.6084/m9.figshare.24411913.v3>. The genome index files were generated with `bismark_genome_preparation` command from Bismark (v0.23.1). Then, alignments were performed using the `bismark` command with default parameters. Finally, methylation information was extracted using `bismark_methylation_extractor` command with the following parameters: `-p -no_overlap -comprehensive -report -bedGraph -CX_context -parallel 20 -cytosine_report`. Differential methylation analysis was identified using the DMRcaller package (v 3.18) within 100 bp windows. DMRs were defined based on thresholds of 40% difference for CG, 20% for CHG, and 10% for CHH.

RNA extraction and quality control

Total RNA was extracted using a combination of TRIzol and the RNeasy® Plant Mini Kit (QIAGEN, Cat.No.74904). RNA quantity and purity were assessed using NanoDrop ND-1000 (NanoDrop, Wilmington, DE, USA). RNA integrity was further evaluated using Bioanalyzer 2100 (Agilent, CA, USA) and verified by agarose gel electrophoresis. RNA samples meeting the following quality criteria were used for downstream experiments: concentration $>50\text{ ng}/\mu\text{L}$, RIN value >7.0 , and $\text{OD}_{260}/280 >1.8$.

Illumina RNA-seq library preparation and transcriptome analysis

RNA-seq libraries were sequenced using Illumina Novaseq 6000 sequencing platform. To ensure high-quality downstream analyses, raw reads containing adapter sequences and low-quality bases were filtered using the `ht2-filter` tool in the HTQC package and `fastp` with default parameters. Subsequently, clean reads were mapped to *D. latiflorus* genome using `Hisat2` (v2.1.0) with default parameters (Kim et al. 2019). The resulting mapped reads were filtered to retain reads aligned to a unique region in the genome using `htslib` 1.9 and quantified using `featureCounts` (Liao et al. 2014). Gene expression levels were normalized as Fragments Per Kilobase per Million mapped reads (FPKM). Differential expressions were analyzed using `DESeq2` (v3.18) (Love et al. 2014). GO enrichment analysis was carried out with `clusterProfiler` (v4.0.2).

Nanopore transcriptome libraries and RNA methylation analysis

DRS libraries were constructed using the Nanopore DRS kit (SQK-RNA002, Oxford Nanopore Technologies). Poly(A)+ RNAs were ligated with ONT's official universal reverse transcription adapter. After the cDNA first strand synthesis and adapters ligation, libraries

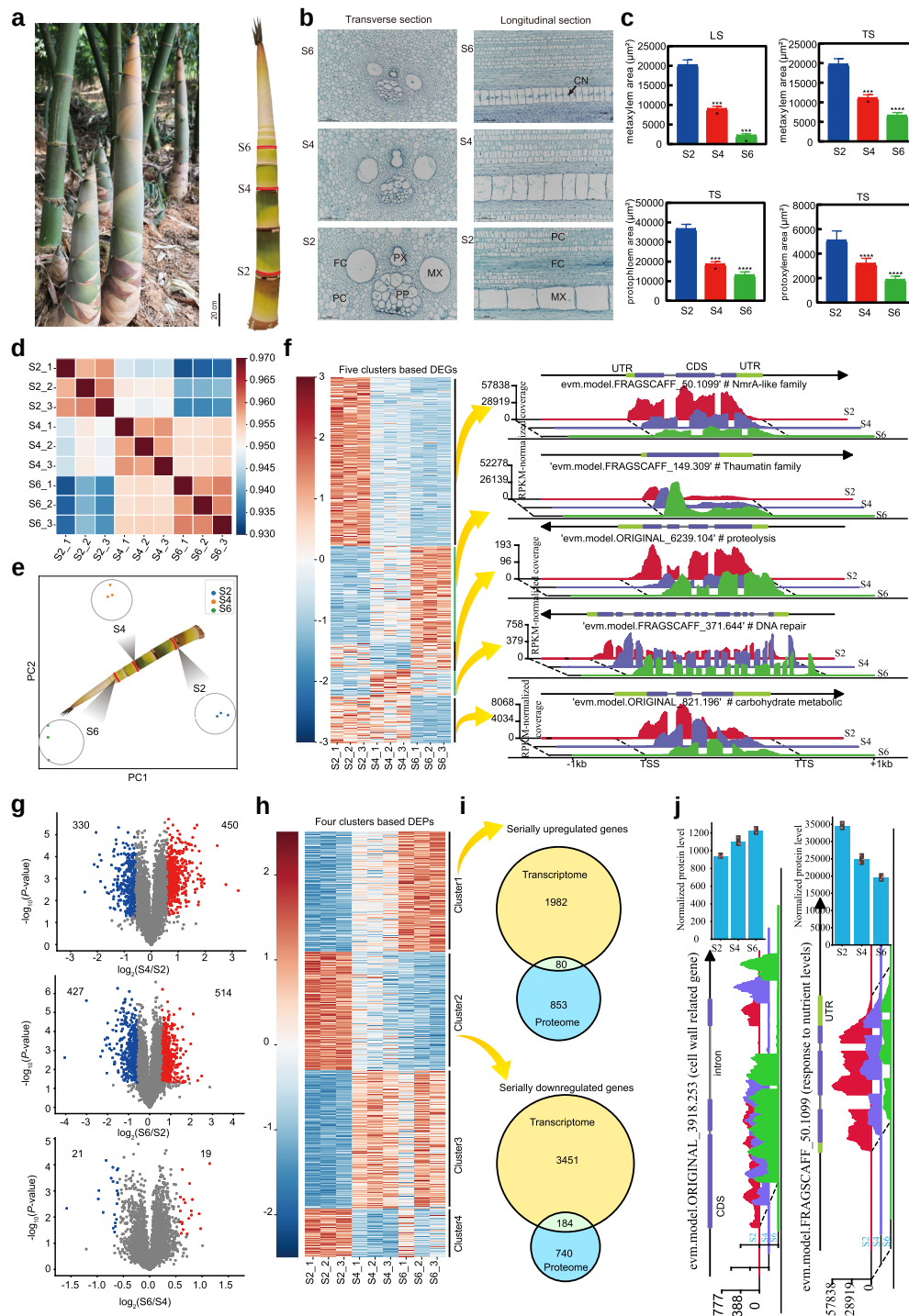


Figure 1 Integrated analysis of morphology, anatomy, transcriptomic, and proteomic dynamics across developmental internodes in Ma bamboo shoots. **a**) Representative shoot showing developmental gradient from the mature base to the juvenile apex. The lines indicate the 3 sampled internodes: differentiated basal (S2, second internode), transitional (S4, fourth internode), and proliferative apical (S6, sixth internode). Scale bar: 20 cm. **b**) Comparative transverse (TS) and longitudinal (LS) sections of at 3 developmental stages. Key anatomical structures are labeled: CN, cell nucleus; FC, fiber cells; MX, metaxylem; PC, parenchyma cells; PP, protophloem; PX, protoxylem. Scale bar: 100 μm . **c**) Quantitative comparison of metaxylem, protophloem, and protoxylem area. Error bars indicate standard deviation. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$ (2-tailed unpaired t test). **d**, **e**) Spearman correlation heatmap (**d**) and principal component analysis (**e**) of transcriptomic profiles across bamboo internodes. **f**) Left panel representing K-means cluster of differentially expressed genes (DEGs) ($|\text{fold change}| > 2$; $P < 0.05$) from pairwise comparisons. The color scale represented z-scores normalized expression level (red: highly expressed gene; blue: lowly expressed genes). Right panel exhibiting genes from each cluster with RNA expression wiggle plots. **g**) Volcano plot showing DEPs ($|\text{fold change}| > 1.5$; $P < 0.05$) between internodes. **h**) K-means clustering heatmap of differentially expressed proteins (DEPs) from pairwise comparisons. Color scale shows z-scores normalized protein abundance (red: high abundance; blue: low abundance). **i**) Venn diagrams showing the overlap between transcriptomic and proteomic datasets. The upper panel indicates serially upregulated genes, and the lower panel shows serially downregulated genes. **j**) Wiggle plots and bar charts showing transcriptomic and proteomic changes for representative genes. Protein data are shown as mean $\pm$ SD ($n = 3$ biologically independent samples).

were sequenced on nanopore platforms. Raw current signals were basecalled using Guppy (v3.6.1). To improve base accuracy, the DRS data was corrected using short reads obtained from Illumina RNA-seq data via LoRDEC.

We identified m⁶A modification at the single-base level using Nanom⁶A (Gao et al. 2021). In short, to ensure compatibility with downstream Tombo analysis, the multi_to_single_fast5 tool was used to split each fast5 file into single-read levels. Raw signals were re-assigned to corresponding transcriptome reference using Tombo's Re-squiggle algorithm. Subsequently, the post-resquiggle signals were extracted to identify m⁶A modification sites.

Poly(A) tail length analysis

Raw DRS current signals from each read were analyzed using Nanopolish (v0.13.2) to estimate the PALs (Parker et al. 2020). For downstream analysis, only reads with a quality control (QC) label of "PASS" in the Nanopolish output were retained. FeatureCounts (v2.0.1) with long-read mode was used to assign each read to annotated genes and only reads labeled as "Assigned" were used for downstream analysis. The PAL of each gene was defined as the median of all reads under this gene that were successfully assigned by FeatureCounts and have a "PASS" QC label from Nanopolish.

Protein extraction and proteome sequencing

Proteins were extracted from bamboo shoots internode tissues for TMT-based quantitative proteomics. Raw spectrum files (.raw) from Q Exactive Plus were converted into .mgf files using Proteome Discoverer 2.1 (Thermo Scientific™) and submitted to the MASCOT 2.6 server for database searching. The resulting search files (.dat) from the MASCOT server were then imported back into the software, and the data were filtered based on FDR < 0.01 to obtain highly reliable qualitative results. The secondary mass spectrometry data were analyzed using MaxQuant (v1.5.2.8). The quantification method was set to TMT-6plex, and both protein identification and PSM identification FDR were set to 1%.

Chromatin immunoprecipitation sequencing

To assess DNA quality, ChIP-DNA degradation and contamination were first assessed using agarose gel electrophoresis. Purity of ChIP-DNA was measured using a NanoPhotometer spectrophotometer (IMPLEN, CA, USA). ChIP-DNA concentration was determined using the Qubit 3.0 Fluorometer (Life Technologies, CA, USA) with the Qubit dsDNA HS and BR Assay Kits (Invitrogen, Cat. No. Q32851). The size distribution of ChIP-DNA fragments was analyzed using the Agilent Bioanalyzer 2100 system, ensuring fragment sizes fell within the 100–500 bp range with a clear dominant band.

Purified DNA was used to construct ChIP-seq library with the NEB Next Ultra II DNA Library Prep Kit for Illumina (NEB, Cat. No. #E7645). The library was then sequenced on the Illumina sequencing platform (Illumina, CA, USA) with paired-end sequencing (PE150) reads. Clean data were aligned to *D. latiflorus* genome using Bowtie2 (v2.3.4.3). SAM files were then converted to BAM format using samtools. bamCoverage from deeptools (v3.5.1) was used to normalize BAM files and generate the bigwig files, which were then converted to wig format using bigWigToWig software for visualization. Peak calling was performed using MACS2 (v2.2.7.1) to identify H3K27me3 and H3K36me3 peaks.

Chromatin accessibility sequencing and processing

Plant samples were flash-frozen in liquid nitrogen and then ground into a fine powder using a TissueLyser II. The powder tissue was re-suspended in pre-chilled Nuclei Purification Buffer, homogenized, and filtered through a cell filter, and centrifuged to separate the filtrate. The pellet was washed sequentially with prechilled Nuclei Extraction Buffer 2 and 3, resuspended, and repeated 3 times. Finally, the pellet was resuspended in Nuclei Purification Buffer for nuclear counting. The isolated nuclei were transferred to a new tube, and incubated with transposition reaction mix, at 37 °C for 30 min with gentle mixing several times. Adapter1 and Adapter2 were then added for PCR amplification. The amplified library was purified using AMPure XP Beads, and DNA concentration and quality were measured using the Qubit dsDNA HS and BR Assay kits. Qualified libraries were sequenced on the Illumina platform using PE150 paired-end sequencing.

Data visualization

All data visualization were generated using the Python programming language, primarily utilizing the matplotlib (version 3.7.1) and seaborn (version 0.11.2) packages in Python (version 3.9.15).

Results

Anatomical characterization and integrated transcriptome-proteome dynamics for developmental gradient in bamboo shoots

In this study, we systematically numbered the internodes of bamboo shoots from the base to the apex (Fig. 1a) and performed cytological sectioning at 3 representative positions: S2 (basal position), S4 (middle position), and S6 (apical position) (Fig. 1b). Longitudinal sections revealed a clear developmental gradient: as the internode position ascended from S2 to S6, metaxylem cells gradually decreased in size and became more densely arranged and exhibited increased cell division activity (Fig. 1b). In S2, metaxylem cells were larger and loosely arranged, indicating a late stage of development. In contrast, S6 displayed smaller, and compactly arranged metaxylem cells. Numerous nuclei were visible in the metaxylem, parenchyma, and fiber cells, reflecting vigorous cell division in S6 (Fig. 1b). Quantitative analysis of transverse sections revealed that the cell size of metaxylem, proto-phloem, and protoxylem presented a gradual decrease from S2 through S4 to S6 (Fig. 1c).

To further explore the molecular basis underlying the observed cellular differences, we performed transcriptomic and proteomic analyses of 3 distinct internode regions (S2, S4, and S6) to assess gene and protein expression profiles (Table S1). Spearman correlation analysis revealed that S4 and S6 exhibited stronger expression similarity with each other than with S2. Furthermore, high reproducibility was observed across replicates (Fig. 1d). This stage-specific pattern was further supported by principal component analysis, which showed tight clustering of biological replicates according to developmental stage (Fig. 1e). We subsequently identified 5 distinct clusters of differentially expressed genes (DEGs) (Fig. 1f). Cluster 1 comprised genes whose expression progressively decreased from S2 (highest) to S4 to S6 (lowest), whereas Cluster 2 included genes with expression levels that increased from S2 (lowest) to S4 to S6 (highest). Gene Ontology (GO) enrichment analysis revealed that S2 internodes were enriched

in cell wall-related processes (eg, cytoskeleton, xylosyltransferase activity, cellulose synthase activity), consistent with their role in providing structural support after cell proliferation (Fig. S1 and Table S2). Conversely, S6 internodes were enriched for genes involved in DNA replication/repair and transcription, reflecting their active cell division at the shoot tip (Fig. S2; Table S2).

To investigate whether these transcriptional patterns were consistent at the protein level, we next performed quantitative proteomic analysis. The proteomic profiles revealed that both S4 and S6 internodes presented a greater number of upregulated than downregulated proteins compared with S2, with minimal differences between S4 and S6 (Fig. 1g). Cluster analysis of the differentially expressed proteins (DEPs) identified 4 major expression patterns (Fig. 1h): (1) proteins progressively upregulated from S2 to S6, (2) proteins progressively downregulated, (3) proteins highly expressed in S4 and S6, and (4) proteins predominantly expressed in S2. A total of 80 genes were serially upregulated at both the transcriptomic and proteomic level, whereas 184 genes were serially downregulated (Fig. 1i). For example, a gene involved in organic cyclic compound binding gene-associated with nutrient response showed a gradual decrease from S2 to S6 at both RNA and protein levels, whereas cell wall related gene showed a progressive increase across the same gradient (Fig. 1j).

DNA methylation landscapes and transcriptional associations across internodal gradients in bamboo shoot

The transcriptional upregulation of several DNA methylation-related genes, including *DOMAINS REARRANGED METHYLTRANSFERASE1* (*DlaDRM1*), *METHYLTRANSFERASE 1* (*DlaMET1*), *Argonaute 4* (*DlaAGO4*), and the demethylase gene *REPRESSOR OF SILENCING 1* (*DlaROS1*), in stage S6 relative to S2 and S4, suggested a potential role of epigenetic regulation via DNA methylation in shoot development (Fig. 2a). To comprehensively characterize DNA methylation across developmental stages, we performed whole-genome bisulfite sequencing (BS-seq) on internodes S2, S4, and S6. Spearman correlation analysis of DNA methylation revealed the highest coefficient between S2 and S4, and the lowest between S2 and S6, with CG/CHG contexts exhibiting stronger correlations than CHH methylation (Fig. 2b). Overall, methylation level of transposable elements (TEs) increased progressively from S2 to S6, with a more pronounced trend in the 1-kb flanking regions upstream and downstream of TEs (Fig. 2c). DNA-type TEs, however, did not show substantial differences in CHH methylation across the 3 internodal stages. In protein-coding genes, CG and CHG methylation levels were higher in S6 than in S4 and S2 (Fig. 2d). CHH methylation levels were higher in both S4 and S6 relative to S2. The distribution of differentially methylated regions (DMRs) across gene features was relatively uniform for CG and CHG contexts, whereas CHH DMRs was enriched in promoters and transcription termination regions (Fig. 2e and f; Table S3), suggesting regulatory specificity of CHH methylation.

Across both CG and CHG contexts, non-expressed genes exhibited higher methylation levels than expressed genes (Fig. 2g). Among the expressed genes, gene-body CG methylation levels gradually increased with rising expression, whereas CHG methylation progressively decreases, reflecting distinct methylation dynamics associated with transcriptional activity. CHH methylation was generally low within gene bodies but enriched in region approximately 100 to

400 bp upstream of transcription start site (TSS). Notably, promoter-specific CHH methylation accumulated progressively with increasing gene expression, while gene bodies maintained a hypomethylated state, highlighting a potential regulatory role of promoter-specific CHH methylation in transcription activation. A similar association pattern between gene expression and DNA methylation context was observed in both S4 and S6 (Fig. 2h).

Histone modification and chromatin accessibility profiling in internodes of bamboo shoots

Functional annotation of clusters 1 and 2 (Fig. 1h) revealed that the up-regulated group was particularly enriched in histone modification-related functions, suggesting a potential role for epigenetic regulation during bamboo shoot development (Fig. S3; Table S2). Especially, we observed a progressive increase in the expression of the histone methyltransferase *SET domain group protein 724* (*SDG724*) from stages S2 to S6, accompanied by a concomitant decrease in the histone demethylase *Jumonji C domain-containing protein* (*JMJ710*) (Fig. 3a). This reciprocal expression pattern suggests a potential shift in the histone modification landscape during shoot developmental stages. To directly test this hypothesis, we performed chromatin immunoprecipitation sequencing (ChIP-seq) to profile 2 critical histone marks: the repressive H3K27me3 and the active H3K36me3. Both H3K27me3 and H3K36me3 peaks were predominantly enriched around TSS (Fig. 3b). In parallel, assay for transposase-accessible chromatin using sequencing (ATAC-seq) revealed that the TSS also exhibited the highest chromatin accessibility (Fig. 3b). Notably, the H3K27me3 level was most abundant in S6, compared with lower levels in S2 and S4, whereas H3K36me3 levels exhibited similar levels in S4 and S6, both of which were higher than in S2 (Fig. 3b). Within TEs, the H3K27me3 and H3K36me3 levels were lowest in S6, whereas S2 and S4 exhibited higher levels (Fig. 3b). Chromatin accessibility was greatest in S6, with S2 and S4 showing reduced openness. Collectively, these findings suggest a developmental shift of chromatin state, driven by histone-mediated regulation and chromatin remodeling.

Pairwise comparisons of histone modification peaks across the 3 developmental stages revealed that the largest number of differential peaks occurred between S6 and S2 (Fig. 3c; Table S4). Using MACS2 with default parameters, we quantified chromatin accessibility peaks in the 3 internode stages. The number of accessible regions increased progressively from S2 (lowest) to S6 (highest), reflecting a developmental enhancement of chromatin openness (Fig. 3d). A total of 3,365 genes harbored accessible chromatin regions shared across all 3 stages (Fig. 3e), while S6 contained the highest number of unique peaks (3,887). Functional enrichment analysis of genes associated with S6-specific accessible regions revealed significant enrichment in pathways related to histone acetylation regulation, antioxidant activity, single-stranded DNA binding, DNA topoisomerase I activity, and phospholipid-transporting ATPase activity (Fig. S4; Table S2). These findings suggest that enhanced chromatin accessibility in S6 is closely associated with regulatory pathways essential for rapid cell proliferation and metabolic activity at the bamboo shoot apex.

Association of chromatin openness and histone modification with gene expression

To explore the relationship between chromatin accessibility and transcriptional activity, genes were ranked according to their

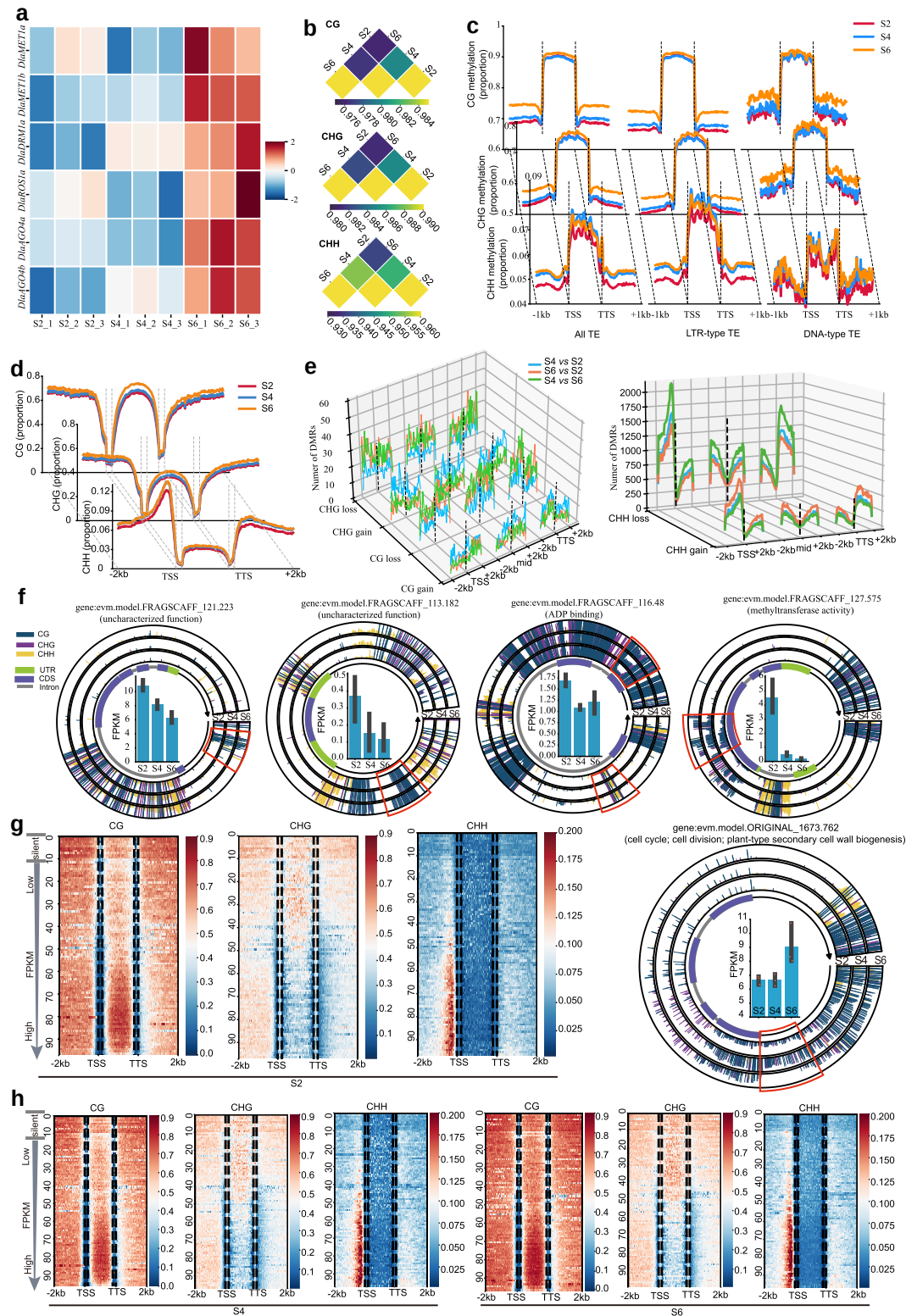


Figure 2 Comprehensive analysis of DNA methylation patterns and genomic distribution. **a**) Expression heatmap of DNA methylation regulatory genes. **b**) Correlation heatmap of CG, CHG, and CHH methylation across samples. **c**) Distribution of DNA methylation within TEs. The 1-kb regions upstream and downstream of each TE were divided into 50 bins, and the TE body was similarly segmented into 50 bins. **d**) Distribution of CG, CHG, and CHH methylation across gene body and 2-kb flanking region. **e**) Distribution of differentially methylated regions DMR around transcription start sites (TSSs), gene bodies midpoints, and transcription termination sites (TTSs) region based on pairwise comparisons. **f**) Wiggle plots depicting differential DNA methylation across 4 genomic loci. **g**) Methylation heatmap across the S2 stage across gene bodies and 2-kb flanking regions. Genes were sorted by expression level (unexpressed genes: top 10 bins; expressed genes: remaining 90 bins). Panels from left to right represent methylation and expression relationships for CG, CHG, and CHH context. **h**) Heatmap showing methylation and expression relationships across gene body and flanking region in S4 and S6 stages.

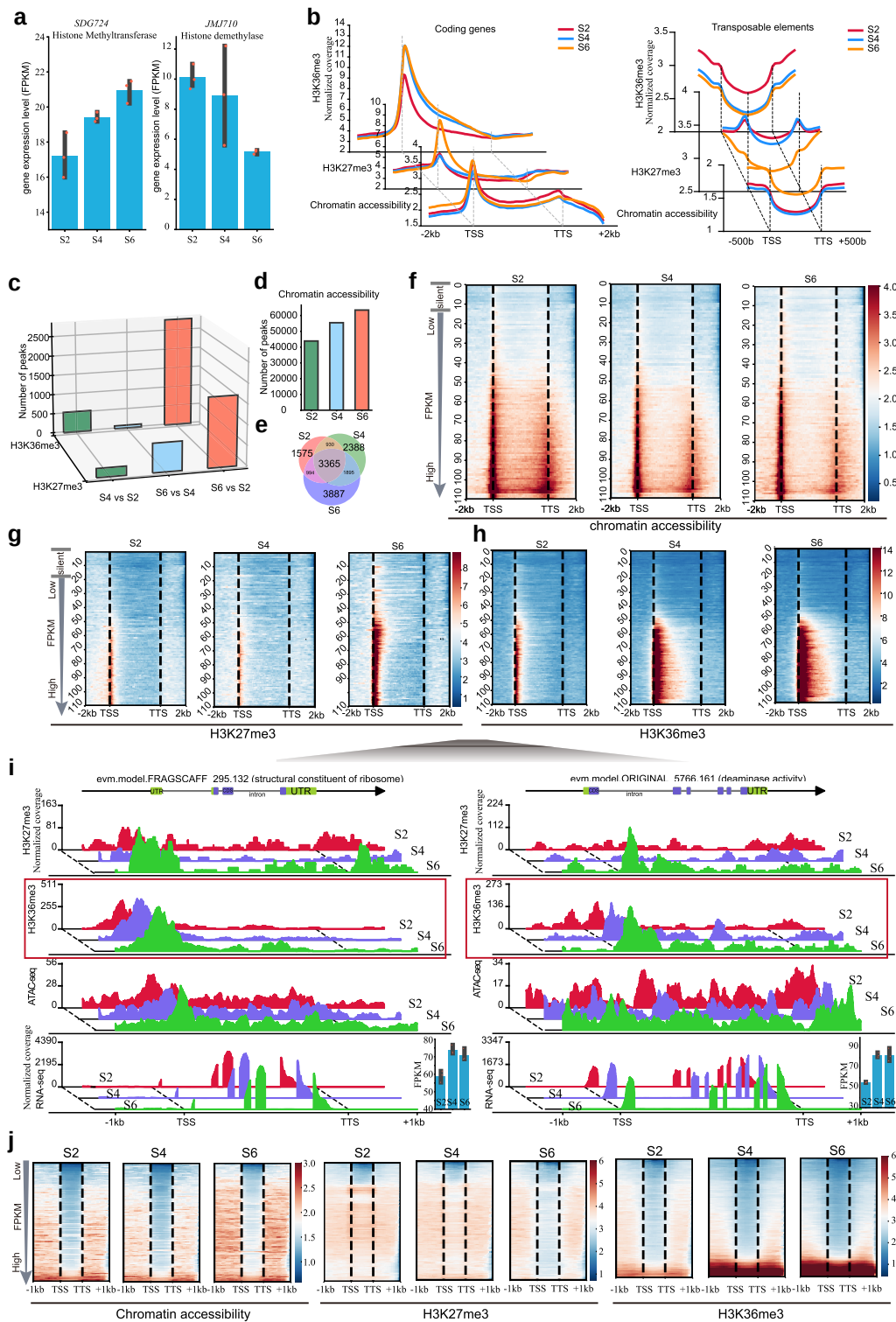


Figure 3 Integrated profiling of chromatin landscapes and associations with genes and transposable element expression during development. **a**) Bar chart displaying the expression levels of *SDG724* and *JMJ710*. Gene expression values are shown as mean $\pm$ SD ($n = 3$ biologically independent samples). **b**) Distribution profiles of chromatin accessibility, H3K27me3, and H3K36me3 signals across coding region (left panel) and TEs (right panel). **c**) Bar chart showing the number of H3K36me3 peaks identified from 3 pairwise comparisons. **d**) Bar chart showing number of accessibility regions in each developmental stage. **e**) Venn diagram illustrates the overlapping of genes associated with accessible chromatin regions among S2, S4, and S6 stages. **f**) Heatmap showing the correlation between chromatin accessibility and gene expression. Genes were grouped into 10 bins (unexpressed) and 100 bins (expressed). Panels from left to right represented S2, S4, and S6, respectively. **g**) Heatmap showing correlation between H3K27me3 levels and genes expression across S2, S4, and S6. **h**) Correlation heatmap between H3K36me3 levels and genes expression across S2, S4, and S6. **i**) Histone modification enrichment profiles at 2 representative loci across S2, S4, and S6 stages. Gene expression values are shown as mean $\pm$ SD ($n = 3$ biologically independent samples). **j**) Heatmap showing the correlation of TE expression with chromatin accessibility (left panel), H3K27me3 levels (middle panel), and H3K36me3 levels (right panel).

expression levels, from lowest to highest. Non-expressed genes exhibited minimal chromatin accessibility signals (Fig. 3f). Chromatin accessibility increased progressively with gene expression, particularly around the TSSs and transcription termination sites (TTS). Highly expressed genes presented broader accessible chromatin regions, characterized by bidirectional extension of open chromatin signal from both TSS and TTS, forming broader accessibility regions. As expected, chromatin accessibility was positively correlated with transcriptional levels, and the overall distribution patterns of chromatin accessibility were consistent across all 3 internodes (Fig. 3f).

For protein coding genes, H3K27me3 modifications were primarily enriched around the TSS (Fig. 3g). Moreover, H3K27me3 signals became detectable only when gene expression exceeded a basal threshold. In highly expressed genes, H3K27me3 enrichment gradually increased around the TSS region. Among the 3 internodes, S2 and S4 exhibited relatively low H3K27me3 level, whereas S6 showed a markedly higher H3K27me3 level (Fig. 3g). H3K36me3 modifications were also primarily enriched around the TSS but showed distinct association patterns with gene activity (Fig. 3h). Non-expressed genes presented minimal H3K36me3 signal, while in S2 internode, H3K36me3 levels maintained low across gene body. However, in S4 and S6 internode, H3K36me3 signal extended from the TSS into gene bodies, coinciding with elevated gene expression levels. This pattern suggests a positive association between H3K36me3 enrichment and transcriptional activity during bamboo shoot development. For example, both histone modification enrichment and transcript levels at representative loci increased progressively from S2 to S6 (Fig. 3i).

For TE-associated genes, we found that elevated TE expression correlated with enhanced chromatin accessibility and a depletion of the repressive mark H3K27me3, particularly at the S6 stage (Fig. 3j). Conversely, highly expressed TEs progressively accumulated the activation-associated mark H3K36me3, particularly at the S4 and S6 stage (Fig. 3j). These results indicate that increased chromatin openness and a shift from H3K27me3 to H3K36me3 deposition facilitates TE transcription activation at the S6 stage.

Cluster-specific epigenetic patterns in bamboo shoot development

To explore the coordinated role of epigenetics factors in internode regulation, we performed clustering analyses using histone modification to generate 10 clusters. Among them the first few clusters presented high expression (Fig. S5a). We focused on Cluster 1–4 due to distinct epigenetic patterns (Fig. 4a and b). In Cluster 1, S6 presented the highest enrichment of both H3K27me3 and H3K36me3 peaks. Chromatin accessibility was concentrated around TSS, with no substantial difference among the 3 internodes. In Cluster 2, H3K27me3 levels were low in S2 and S4 but increased markedly in S6, while H3K36me3 enrichment peaked in both S4 and S6. GO analysis of Cluster 2 genes revealed the term of histone lysine methylation under biological process category (Fig. S5b; Table S2). Gene annotation indicated that these genes contained Pre-SET and SET domains characteristic of histone methyltransferases. Consistently, RNA expression analysis showed their expression levels gradually increased from S2 to S6 (Fig. 4c). Cluster 3 was characterized by consistently low H3K27me3 across all internodes, whereas H3K36me3 levels were elevated around both the TSS and gene body regions, particularly in S4 and S6. Especially,

chromatin accessibility in this cluster was also enriched not only at TSSs but also across gene bodies, indicating a highly active chromatin state. In Cluster 4, H3K27me3 was enriched at TSS, reaching its highest level in S6, whereas H3K36me3 exhibited the strongest signal in S2. Because H3K36me3 modification exhibits a positive correlation with gene expression Cluster 4 genes displayed higher expression level in S2 than in S4 or S6 (Fig. 4d). This consistent pattern between H3K36me3 and expression level is also evident at the individual genes level, where H3K36me3 modification dynamics align with expression changes (Fig. 4e).

Previous studies have demonstrated the tight association between histone modifications and DNA methylation (Dhayan et al. 2010; Stewart et al. 2015). Therefore, we examined DNA methylation change across the 4 major patterns (Fig. 4f). In Cluster 1, genes showed the highest CG methylation level within gene bodies, low CHG methylation, and elevated CHH methylation in promoter regions. Cluster 2 genes showed low CG methylation at TSS/TTS, reduced CHG methylation within gene bodies, along with high CHH methylation in promoters. In Cluster 3, CG and CHG levels followed the genome-wide average (gray line), whereas CHH methylation remained elevated in promoter regions. Cluster 4 exhibited a distinct methylation profile, with both CG and CHG peaked near the TSSs and extending into gene bodies (Fig. 4g), a pattern not observed in Clusters 1–3. Correspondingly, cluster 4 genes showed overall lower expression levels, with higher expression in S2 compared with S4 and S6 (Fig. 4h). In contrast, genes in clusters 1 to 3 maintained high expression levels across internodes. The remaining 6 clusters exhibited overall low expression levels, with no substantial differences in histone modification or DNA methylation patterns among the 3 internodes (Fig. 4i–k).

Given that DNA methylation has been linked to poly(A) tail lengths (PALs) (Li et al. 2023), we next divided the ten gene clusters into 3 positional intervals—the front (0 to 30 bins), middle (30 to 70 bins), and rear (70 to 100 bins) portion. Correlation analysis between CG methylation and PALs revealed a strong positive correlation, particularly pronounced in the rear portion (70 to 100 bins) (Fig. 4l), suggesting that DNA methylation may interplay with PAL modulation in bamboo shoots.

Comprehensive profiling of poly(A) tail lengths and associations with RNA expression during bamboo shoot development

Nanopore DRS provides an effective tool for accurately detecting PAL (Gao et al. 2022). Therefore, we conducted a comprehensive analysis of PAL dynamic across different developmental stages of bamboo shoots. DRS results revealed that average PALs were longer in S4 (78.83 bp) and S6 (72.69 bp) compared with S2 (69.23 bp) (Table S5). Distribution density analysis further showed that S2 transcripts possessed shorter PALs, primarily concentrated in the lower length range, whereas S4 and S6 exhibited broader distributions with longer tails (Fig. 5a), reflecting overall elongation of poly(A) tails. These observations suggest dynamic regulation of PALs during bamboo shoot development. Pairwise comparisons among S2, S4, and S6 also revealed that the majority of transcripts in S4 and S6 exhibited longer PALs than those in S2 (Fig. 5b and c), consistent with global length distribution trends. Venn diagram analysis of transcripts with substantially altered PALs identified 52 overlapping genes with shortened PALs and 720 overlapping genes with elongated PALs (Fig. 5d; Table S5).

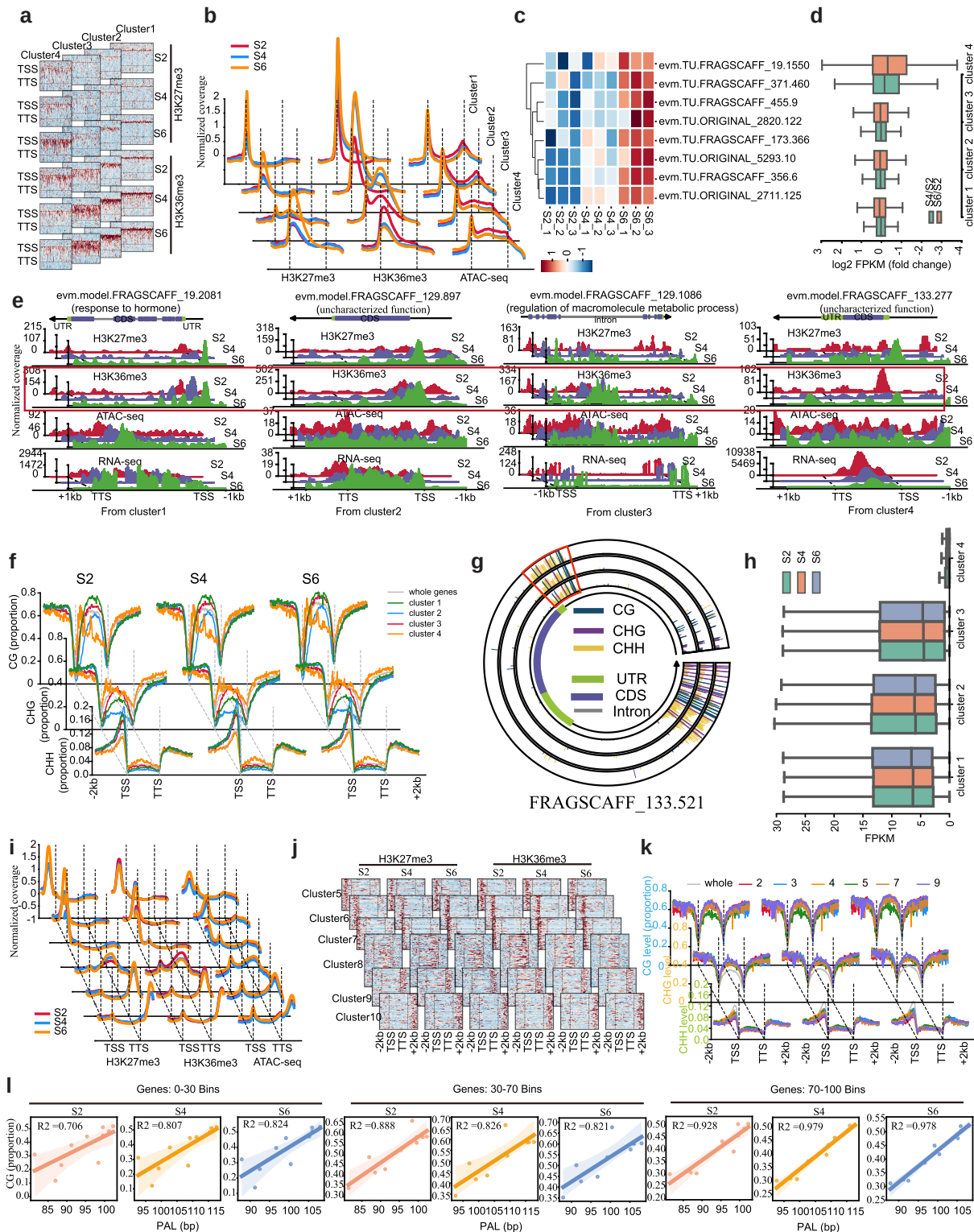


Figure 4 Integrated epigenetic landscape and DNA methylation dynamics across developmental stages. **a)** Heatmap of H3K27me3 and H3K36me3 patterns using K-means clustering. **b)** Overall distribution profiles of epigenetic signals across the 4 K-means categories. **c)** Heatmap showing the expression patterns of genes encoding Pre-SET and SET domains. **d)** Box plot showing corresponding pairwise fold changes of expression (FPKM) among 4 categories. Box limits represent the first (Q1, 25th percentile) and third (Q3, 75th percentile) quartiles; the center line, the median (50th percentile); and whiskers, the most extreme data points within $1.5 \times \text{IQR}$ from the quartiles. **e)** Wiggle plots depicting representative genes from each of the 4 clusters. **f)** The global distribution of CG, CHG and CHH methylation across the 4 categories. **g)** Wiggle plots showing DNA methylation enrichment near TSS. **h)** Bar chart showing gene expression levels (FPKM) for each of the 4 categories. Box limits represent the first (Q1, 25th percentile) and third (Q3, 75th percentile) quartiles; the center line, the median (50th percentile); and whiskers, the most extreme data points within $1.5 \times \text{IQR}$ from the quartiles. **i-k)** Characteristic of 6 additional clusters: (i) distribution of profiles, (j) heatmap of histone modification signals, and (k) DNA methylation pattern. **l)** Correlation between CG methylation and poly(A) lengths (PALs) across ten K-means clusters.

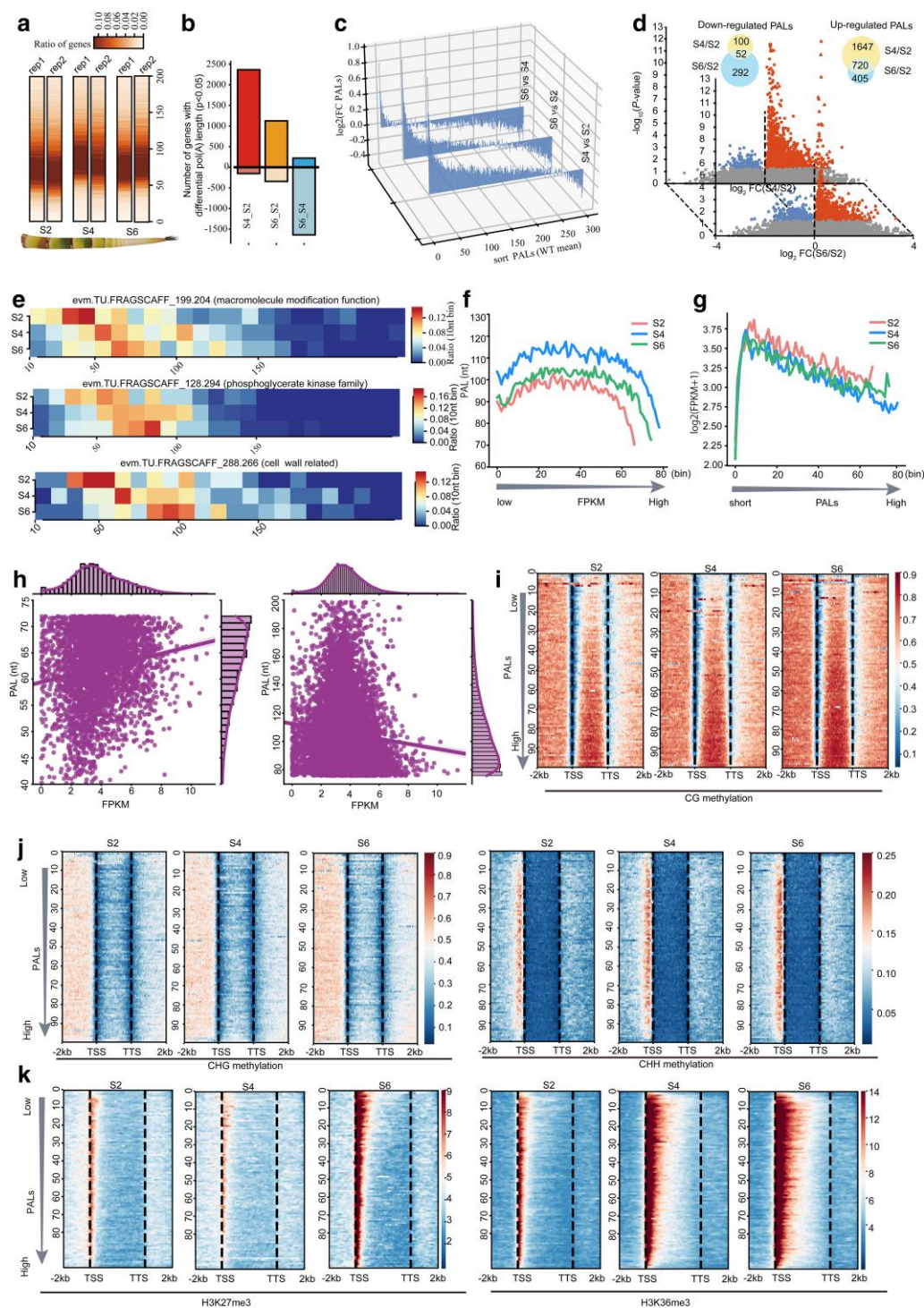


Figure 5 Poly(A) tail length dynamics and associations with gene expression and epigenetic modifications during shoot development. **a**) Heatmap showing median PALs across developmental stages. **b**) Bar plots showing the number of genes exhibiting substantial PAL changes in 3 pairwise comparisons. **c**) PALs Differences across 3 pairwise comparisons. The y-axis represents $\log_2(\text{fold change})$, and the x-axis showing lengths sorted by PALs. **d**) Volcano plot illustrating differential PALs in pairwise comparisons. Genes with longer PALs were shown in right side, and those with shortened PALs in left side (2-tailed Student's t -test, $P < 0.05$). Left panel represented overlapped genes with decreased PALs, and right panel represented overlapped genes with increased PALs. **e**) Heatmap showing 3 representative genes with differential PALs. **f, g**) Correlation between PALs and gene expression (FPKM). In (f), genes are ranked by expression levels on the x-axis, with corresponding PALs shown on the y-axis. In (g), genes are ranked by PALs (x-axis), with corresponding expression levels (y-axis). **h**) Scatter plots showing correlation between PAL and gene expression (FPKM). Left panel shows the top 10% of genes with shortest PALs, presenting positive correlation. Right panel shows the remaining 90% of genes, exhibiting negative correlation. **i**) Heatmap showing correlation between PALs and CG methylation levels. Genes are ranked by PALs and divided into 100 bins from shortest to longest. From left to right, the heatmap shows CG methylation levels in S2, S4, and S6, respectively. **j**) Heatmap showing the correlation between PALs and CHG/CHH methylation. **k**) Heatmap showing H3K27me3 and H3K36me3 enrichment across genes ranked by PALs. From left to right, the heatmap displays enrichment of H3K27me3 and H3K36me3 in S2, S4, and S6.

For example, genes related to macromolecule modification function, phosphoglycerate kinase, and plant-type cell wall, presented marked PAL extension in S4 and S6 (Fig. 5e). GO enrichment analysis of genes with upregulated PALs highlighted translation-related terms, suggesting a potential link between PAL elongation and translational efficiency (Fig. S6; Table S2).

To further explore the relationship between PALs and gene expressions, we ranked genes by expression level and observed that PALs initially increased and but then progressively declined, suggesting a nonlinear relationship between PALs and gene expression (Fig. 5f). Similarly, when genes were ranked by PALs, we found that within the top 10% of genes with relatively short tails, PALs positively correlated with RNA expression (Fig. 5g). Conversely, for the remaining 90% of genes with longer tails, PALs exhibited a negative correlation with expression (Fig. 5g). Notably, this biphasic trend was highly consistent across all 3 internode stages (S2, S4, and S6). Using the inflection point identified above, we constructed separate scatter plots of RNA expression vs PALs for genes partitioned by this threshold. The model fitting also revealed a positive correlation between PALs and transcript abundance among the top 10% of genes with shorter tails, whereas a negative correlation was observed in the remaining 90% with longer tails (Fig. 5h). These findings suggest that PAL exerts dual effects on mRNA expression during bamboo shoot development.

PALs dynamics interlinked with DNA methylation and histone modifications in bamboo shoot development

Across all 3 developmental stages (S2, S4, and S6), we observed a consistent positive correlation between PALs and gene body CG methylation (Fig. 5i). While CG methylation progressive increased in parallel with PALs, neither CHG nor CHH methylation displayed association with PALs variation during the same developmental progression (Fig. 5j). Both H3K27me3 and H3K36me3 histone modifications showed negative correlations with PALs (Fig. 5k), suggesting that higher levels of these modifications may correspond to shorter poly(A) tails during bamboo shoot development. Especially, H3K27me3 levels were relatively low at S2 and S4 but increased markedly at S6, while H3K36me3 displayed increased levels observed in S4 and S6 (Fig. 5k).

Developmental remodeling of the m⁶A epitranscriptome in elongating bamboo shoots

The m⁶A modification is regulated by writer, reader and eraser (Jia et al. 2011; Zhang et al. 2024; Wang et al. 2025a). We found that several m⁶A erasers, including *AlkB homolog 9* (*DlaALKBH9*) and *AlkB homolog 10* (*DlaALKBH10*), were downregulated at S6 stage (Fig. 6a). To complement epigenetic-centric analyses, we explored RNA epigenomic modifications across bamboo internodes by direct sequencing transcriptome-wide m⁶A using Nanopore DRS (Table S6). The highest m⁶A methylation levels were detected at S2 stage, followed by S4, with the lowest levels detected at S6 (Fig. 6b). Furthermore, analysis of RRACH consensus motifs (AAACA, AAAC, GAACA, GAACC, and GAAC) revealed higher m⁶A levels in S2 than in S4 and S6 (Fig. 6c). Among these motifs, AAACA motif exhibited the highest overall m⁶A enrichment (Fig. 6d). Consistent with conserved m⁶A patterns (Li et al. 2023), the density of m⁶A site progressively increased toward the 3' end, where it reached peak (Fig. 6e). Notably, S4 exhibited stronger m⁶A enrichment near the 3' end. Analysis of m⁶A modification levels across gene structures in each bin revealed that m⁶A levels were

higher in both 5' and 3'UTRs than in CDS regions, where methylation was lower with uniform flat distribution curves (Fig. 6f). Globally, S2 exhibited elevated m⁶A levels across all regions compared with S4 and S6.

Clustering of differential m⁶A genes (DMGs) also revealed that the 2 largest clusters exhibited the highest m⁶A levels in S2, whereas the cluster with higher m⁶A levels in S4/S6 accounted for only a small fraction (Fig. 6g). Compared with S2, both S4 and S6 exhibited reduced m⁶A level. Specifically, S4 contained 1,547 hypo-methylated sites and 211 hyper-methylated sites, while S6 showed 1,395 hypo-methylated sites and 157 hyper-methylated sites (Fig. 6h; Table S6). Overlapping analysis of DMGs between S4 and S2 and between S6 and S2 revealed a higher proportion of overlapping hypomethylated genes. GO enrichment analysis of the 630 overlapped hypomethylated genes identified significant functional categories related to growth processes (Figs. S7–S9; Table S2). In molecular functions category, protein-related activities were prominent, including translation elongation factor activity, ribosome binding, and unfolded protein binding. Additionally, key energy metabolism enzymes were also identified, such as malate dehydrogenase activity, fructose-bisphosphate aldolase activity, and oxidoreductase activities. Notably, structural constituents of cytoskeleton emerged as an important function, maintaining cellular architecture during rapid growth. In the cellular components category, significant enriched terms included nucleosome organization and mitochondrial proton-transporting ATP synthase complex.

DEGs analysis of the 630 overlapped hypomethylated genes revealed that in the S4 versus S2 comparison, 320 genes were upregulated and 96 were downregulated (Fig. 6i). In the S6 versus S2 comparison, 278 upregulated and 154 downregulated genes were identified. These results demonstrated a general association between reduced m⁶A methylation and corresponding increased RNA expression across developmental stages. As illustrated in the 2 representative examples, a negative correlation between m⁶A modification and expression was observed across 3 tissues (Fig. 6j).

A previous study revealed the link between m⁶A and PALs (Zhang et al. 2024). In this study, we observed a positive correlation between PALs and m⁶A levels (Fig. 6k). The most pronounced increase in m⁶A occurred at S2 as poly(A) tails extended. When m⁶A levels reached the top 40% PALs in S2, PALs exhibited exponential growth, whereas in S4 and S6, PALs increased linearly. It will be interesting to investigate why the regulatory relationship between PAL and m⁶A during the S2 phase differs from that in other periods in the future.

Discussion

The rapid growth of bamboo shoots is a distinctive biological characteristic of bamboo species. Bamboo shoots not only possess economic value (Jiang et al. 2024) but also provide valuable insights into enhancing crop yield from a molecular biology perspective. For example, the demethylase FTO plays important role in enhancing rice yield (Jia et al. 2011). Epigenetic regulatory in running bamboos, such as *P. edulis* has been investigated in previous studies (Li et al. 2023; Xi et al. 2023). However, the epigenetic and post-transcriptional regulatory of clumping bamboos such as *D. latiflorus* remained largely unknown. Thus, this study presents a multi-omics characterization of shoot development, focusing on the integrated epigenetic and post-transcriptional regulatory landscapes that shape internode elongation. By integrating cytological, transcriptomic,

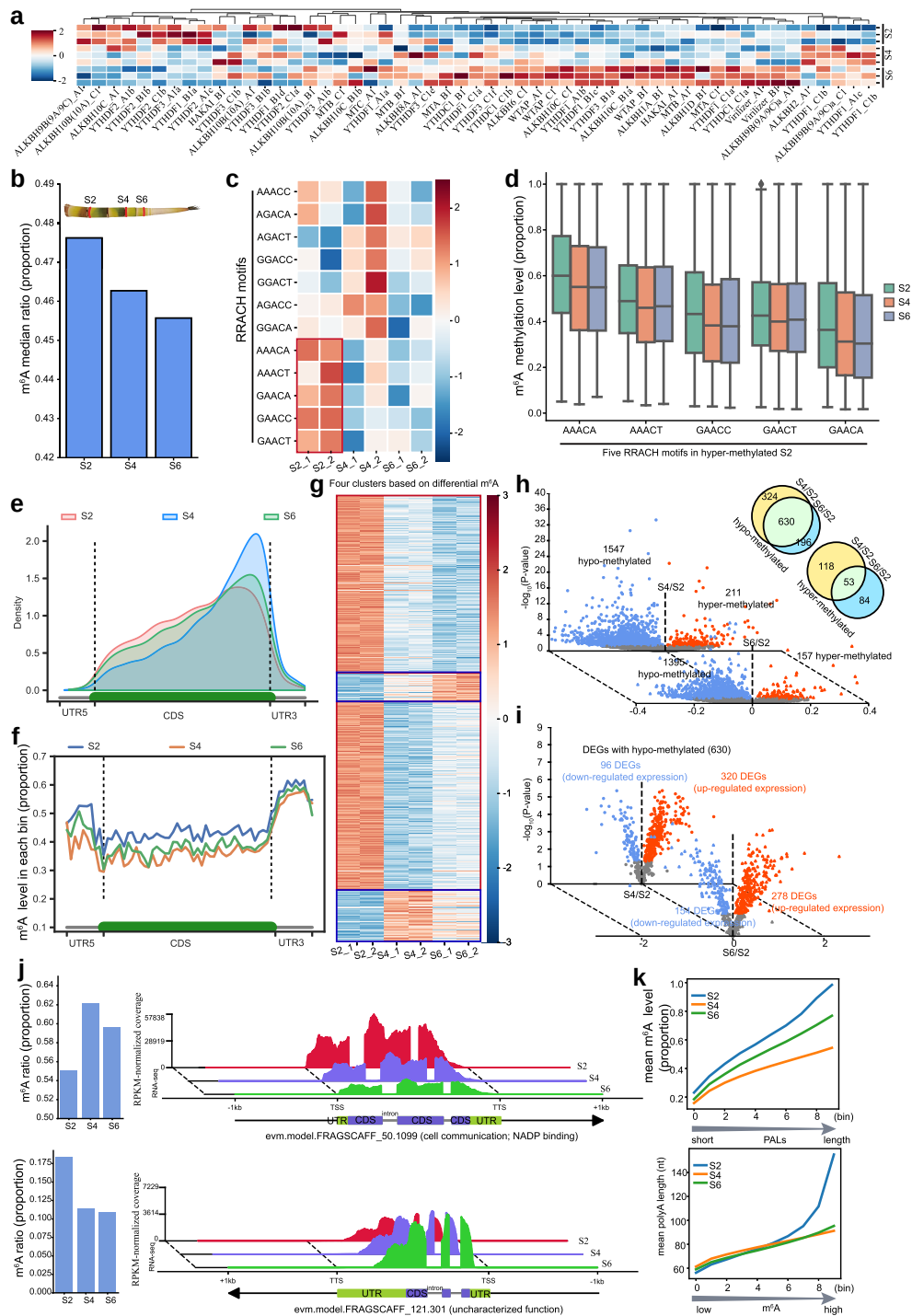


Figure 6 Comprehensive profiling of m^6A methylation dynamics and its functional interplay with gene expression and PALs during development. **a)** Heatmap showing expression of m^6A writer, eraser, and reader in S2, S4, and S6. **b)** Bar plot showing global m^6A levels across developmental stages. **c)** Heatmap showing m^6A level across 12 modification motifs. **d)** Box plot displaying overall m^6A modification levels for 5 RNA modification motifs in S2, S4, and S6. Box limits represent the first (Q1, 25th percentile) and third (Q3, 75th percentile) quartiles; the center line, the median (50th percentile); and whiskers, the most extreme data points within $1.5 \times IQR$ from the quartiles. **e)** Density distribution of m^6A sites across genic features (UTR and CDS). **f)** Level of m^6A modification across genic regions. The 5' UTR and 3' UTR were each divided into 10 bins, while CDS were divided into 40 bins. Average m^6A methylation levels were calculated and visualized per bin. **g)** Heatmap showing clusters of differential m^6A modification. **h)** Volcano plots showing differential m^6A based on pairwise comparisons (S4 vs S2 and S6 vs S2). Hyper-methylated sites are shown in right side, and hypo-methylated sites in left side (2-proportion Z-test, $P < 0.05$). Venn diagrams show overlapping genes in differential m^6A comparisons. **i)** Volcano plots showing DEGs among the genes with hypo-methylated sites. Upregulated genes are shown in right side, and downregulated genes in left side ($P < 0.05$). **j)** The bar chart in the left panel displaying the m^6A modification levels, while the wiggle plot in the right panel showing the gene expression levels at S2, S4, and S6. **k)** Correlation between m^6A levels and PALs across developmental stages. The upper panel plots m^6A levels (y-axis) against genes ranked by increasing PALs (x-axis); the bottom panel plots PALs (y-axis) against genes ranked by increasing m^6A levels (x-axis). In both panels, the x-axis is divided into ten equal groups (deciles).

proteome sequencing, epigenomic (BS-seq, ATAC-seq, H3K27me3, and H3K36me3), and epitranscriptomic data (Nanopore DRS) from 3 distinct internode stages (S2, S4, and S6), we uncovered how chromatin state, DNA methylation, histone modifications, RNA methylation, poly(A) tail dynamics, and protein abundance collectively modulate shoot growth in *D. latiflorus*.

Cytological changes coincidental with transcriptomic profiles

Anatomical observations in *D. latiflorus* revealed a progressive transition from structural reinforcement in basal internodes to proliferative activity in apical internodes, similar to that observed in *P. edulis* shoots during fast growth (Wang et al. 2021; Yang et al. 2021). These cytological changes coincidental with transcriptomic profiles, which showed enrichment of cell wall biosynthesis genes in S2 and increased expression of DNA replication/repair and transcription-related genes in S6, consistent with enhanced cell division activity. DEGs analysis revealed key epigenetic regulators (Figs. 2a, 3a, and 6a) that might contribute to stage-specific functional transitions and thus pointed to as a major contributor to shoot development. Therefore, we next focused on several epigenetics features underlying bamboo shoot growth.

DNA methylation dynamics and context-dependent roles in transcriptional regulation

Genome-wide bisulfite sequencing revealed DNA methylation dynamics changes across internodes (Fig. 2c–e). Gene body CG and CHG methylation increased with apical development, particularly in S6. Both DNA methyltransferases and demethylases were concurrently upregulated in S6 (Fig. 2a), suggesting a balanced regulation that might collectively determine the final methylation state. The association between methylation and transcriptional activity was context-dependent: positive for CG (gene bodies) and CHH (promoters), but negative for CHG (gene bodies) (Fig. 2g and h). These findings indicate distinct functional roles of CG, CHG, and CHH methylation in transcriptional activation and repression, consistent with patterns reported in *P. edulis* (Zhang et al. 2021).

H3K36me3 dynamics as a key positive regulator of gene expression during development

H3K36me3 is a well-established histone marker of active transcription (Yao et al. 2025). Histone modification profiling highlighted its contribution to gene activation in *D. latiflorus*. H3K36me3 levels varied substantially across S2, S4, and S6, showing a strong positive correlation with gene expression levels (Fig. 3h). H3K36me3 increased from S2 to S4/S6, particularly within gene bodies, mirroring transcriptional activation patterns. In contrast, H3K27me3, a repressive mark (Cai et al. 2021), was also elevated in S6 relative to S2 and S4, suggesting fine-tune cell differentiation during rapid growth. However, the global H3K27me3 level remained relatively stable between S2 and S4, indicating a more modest impact on gene expression compared with H3K36me3.

Stage-specific chromatin accessibility and histone modification of transposable elements during shoot growth

ATAC-seq data revealed highest chromatin accessibility near TSS regions across all stages, with increased openness in S6 (Fig. 3j). TE expression positively correlated with flanking chromatin accessibility, especially in S6, suggesting a more permissive chromatin environment for TE activity. Although a small subset of TEs exhibits higher H3K36me3 in S6 (Fig. 3j), this group represents only a minor fraction of all expressed TEs. Consequently, the genome-wide average pattern remains dominated by higher H3K36me3 in S2 (Fig. 3b). We classified TEs into high- and low- H3K36me3 groups (Fig. S10). The high-H3K36me3 group indeed shows the highest H3K36me3 in S6, whereas the low-H3K36me3 group exhibits the highest H3K36me3 in S2, consistent with the overall trend in Fig. 3b.

Combined with reduced H3K27me3 and increased H3K36me3 levels within TEs in S6, these findings indicate stage-specific regulation of TEs during shoot elongation. To investigate whether active TEs influence gene differential gene expression, we analyzed the distribution of TEs around all DEGs (Fig. 1f, clusters 1 and 2). DEGs were categorized based on the presence of TEs within 3 genomic contexts: the promoter region (2 kb upstream), intragenic regions (introns), and downstream of the transcription termination site (2 kb downstream). The results revealed that only a minimal proportion of DEGs were associated with nearby active TEs (Fig. S11), suggesting that these active TEs are predominantly located in distal to DEGs. The functional significance and precise role of these active TEs in internode development remains to be elucidated and warrants further investigation.

Poly(A) tail length dynamics reveal biphasic expression regulation

PAL analyses uncovered developmentally regulated extension of PALs, with longer tails in S4 and S6 compared with S2 (Fig. 5a–d). Interestingly, a biphasic relationship between PAL and RNA expression emerged: within the top 10% of transcripts with shorter PALs, expression positively correlated with tail length. Beyond this subset, PALs showed a negative correlation with expression (Fig. 5g). The previously unreported was highly conserved across all internodes, suggesting that poly(A) tail regulation may exert dual effects on mRNA abundance, a compelling hypothesis for further investigation.

To explore the association between intronic TEs and PAL, we first classified genes into 3 categories based on TE location: TE-depleted genes, 3'-proximal TE-containing genes (TEs located within the 2 kb region downstream of the transcription termination site), and intronic TE-containing genes. Box plots of PALs for these 3 groups showed that in all samples (S2, S4, and S6), genes containing intronic TEs exhibited a higher median PALs compared with the other categories (Fig. S12a), suggesting that the presence of intronic TEs may lead to increased PAL. We further subclassified the intronic TE-containing genes based on the relative position of the TE within the gene body: 5'-proximal (first 30%), central (30–70%), and 3'-proximal (last 30%). We observed a obvious increase in the median PALs specifically when TEs were located in the 3'-proximal region (Fig. S12b). These results suggest that TEs positioned near the end of a gene may promote lengthening of the poly(A) tail. As the current evidence remains correlative, future experimental work is needed to elucidate the underlying regulatory mechanism.

Global decline and spatial redistribution of m⁶A underlies its role in growth-stage transitions

Nanopore DRS enables the potential for the accurate detection of RNA modifications and PAL (Gao et al. 2022). DRS revealed a global decline in m⁶A levels from S2 to S6 (Fig. 6b). This trend was motif-specific, with RRACH motifs-particularly AAACA-showing strongest enrichment in S2 (Fig. 6d). The downregulation of 2 RNA demethylases, DlaALKBH9 and DlaALKBH10 (Fig. 6a), may explain the observed global decrease in methylation. The m⁶A distribution along transcript also shifted, with S4 displayed a 3'-end bias (Fig. 6e). This spatial shift likely reflects dynamic changes in the binding sites of RNA methyltransferases and demethylases across different stages. Functionally, hypo-methylated genes were enriched for growth-related processes, and a subset of them showed upregulated expression (Fig. 6h-j), supporting the idea that m⁶A may act as a suppressive modification at early stage (S2), lifted to promote growth in later stages (S6).

Previous studies have revealed the association between exon junction complexes (EJCs) and m⁶A. To explore this relationship in relation to gene structure, we first investigated the m⁶A levels in single-exon genes. The results showed that in genes with only 1 exon, the 3' UTR and 5' UTR still exhibited higher m⁶A levels than the CDS region (Fig. S13). In multi-exon genes, the internal exons displayed a slightly higher overall m⁶A level compared with the first and last exons, although the difference was subtle. Due to potential detection biases introduced by DRS depth in third-generation sequencing, further investigation using second-generation-based m⁶A detection methods in the future will be valuable for exploration of the relationship between EJCs and m⁶A levels.

Crosstalk between epigenetic and post-transcriptional layers

Our multi-omics datasets provided a comprehensive view of the dynamic of gene expression, DNA methylation, histone modifications, PAL, and RNA modifications during the development of individual bamboo internodes. Since these regulatory layers operate interdependently, we investigated their crosstalk in detail. Firstly, we found that PALs positively correlated with CG methylation within gene bodies (Figs. 4l and 5i) but showed no correlation with CHG or CHH methylation (Fig. 5j). Furthermore, both H3K27me3 and H3K36me3 were negatively correlated with PALs (Fig. 5k), suggesting that transcripts originating from chromatin regions enriched for histone marks tend to have shorter poly(A) tails. The potential interplay between chromatin state and post-transcriptional regulation warrants further investigation. Finally, PALs positively correlated with m⁶A levels, particularly in S2, where exponential tail elongation occurred in highly methylated transcripts (Fig. 6k). Together, these relationships reveal coordinated yet complex interactions between chromatin state and RNA metabolism, potentially enabling fine-tuning of gene expression control during rapid tissue expansion.

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Author contributions

L.G conceived this project and designed the experiments. Z.Z., T.H., J.Z., and H.W., performed bioinformatics analysis. L.W., D.Y., Y.Y., L.X., L.Z., H.Z., and Y.Z carried out the experiments. Z.Z., and L.G wrote the manuscript. All authors contributed to the article and approved the submitted version.

Supplementary material

Supplementary material is available at *Plant Physiology* online.

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Conflict of interest

The authors declare that they have no competing interests.

Data availability

Sequence data from this article can be found in the National Genomics Data Center (NGDC) under accession numbers CRA017879.

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