

Review

Advances of N⁶-methyladenosine RNA methylation in tree species

Huihui Wang (王慧慧)^{1,#}, Huiyuan Wang (王汇源)^{2,3,#}, Xiaoxia Jin (靳晓霞)¹, Yusen Wang (王雨森)⁴, Liangzhen Zhao (赵良真)⁴, Bochen Jiang (姜博晨)^{2,3}, and Lianfeng Gu (顾连峰)^{5,*}

N⁶-methyladenosine (m⁶A) is a pivotal post-transcriptional regulator in plants, but most mechanistic studies derive from arabidopsis (*Arabidopsis thaliana*) and annual crops. While core m⁶A machinery is conserved in plants, emerging studies in poplar, bamboo, and other tree species suggest that m⁶A modulates lignification and stress adaptation, likely reflecting perennial traits including long life cycle, secondary growth, seasonal developmental transitions. The major challenge of m⁶A research in trees is the shift from global m⁶A profiling to site-specific causal mechanism. New approaches like targets of RNA-binding proteins identified by editing, direct RNA sequencing, and dCas13-based programmable RNA epigenetic editors provide a practical resolution. In summary, this review extends m⁶A regulation from model plants to trees and highlights m⁶A's potential for improving tree traits.

m⁶A RNA methylation in trees

N⁶-methyladenosine (m⁶A) is one of the most prevalent and extensively studied RNA modifications in eukaryotic mRNAs. Its widespread distribution and dynamic regulation are essential for gene expression. In plants, mechanistic research on m⁶A modification mainly derives from model plants, including arabidopsis and rice, revealing that m⁶A modification affects RNA metabolism and is involved in plant growth and development, such as embryonic development [1], leaf morphology [2], the flowering process [3], yield improvement [4], and stress responses [5–8].

However, recent research on tree species indicates that m⁶A modification is associated with xylem development, root formation, and stress responses, which differs from its functions in model plants. This difference may be due to the long life cycle and the unique secondary growth process of trees, which require a complex regulatory system. Considering that previous reviews have extensively covered the conserved principles of m⁶A modification in plants, we focus on the unique features and emerging functions of m⁶A regulation in tree species. We discuss the expansion of m⁶A regulatory network, the role of m⁶A in specific biological processes of trees, and how to advance m⁶A research from correlative findings to site-specific causal validation. By integrating current advances and future perspectives, this review aims to provide a comprehensive perspective and practical guidance for m⁶A modification in tree species.

Regulatory proteins of m⁶A writers, readers, and erasers

The m⁶A modification is dynamically controlled by three classes of proteins: methyltransferases (writers) that install m⁶A, reader proteins (readers) that recognize m⁶A, and demethylases (erasers) that remove m⁶A, collectively referred to as m⁶A regulatory components [9]. Tight cooperation between m⁶A writers and erasers maintains m⁶A homeostasis, which is essential for various biological processes in plants (Figure 1). Compared to model plants like arabidopsis,

Highlights

Core N⁶-methyladenosine machinery is widely conserved in plants, but its biological function appears some specificity in tree species.

Current evidence suggests that N⁶-methyladenosine in tree species is most strongly associated with root development, lignification, and long-term stress adaptation.

Gene family expansion, long life cycles, and transformation barriers increase the mechanistic complexity of m⁶A in trees.

With tools like hyper targets of RNA-binding proteins identified by editing, direct RNA sequencing, and programmable RNA editors, N⁶-methyladenosine research in trees could shift from correlation-based findings toward causal mechanisms.

¹College of Forestry, Fujian Agriculture and Forestry University, Fuzhou 350002, China

²School of Life Sciences & Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China

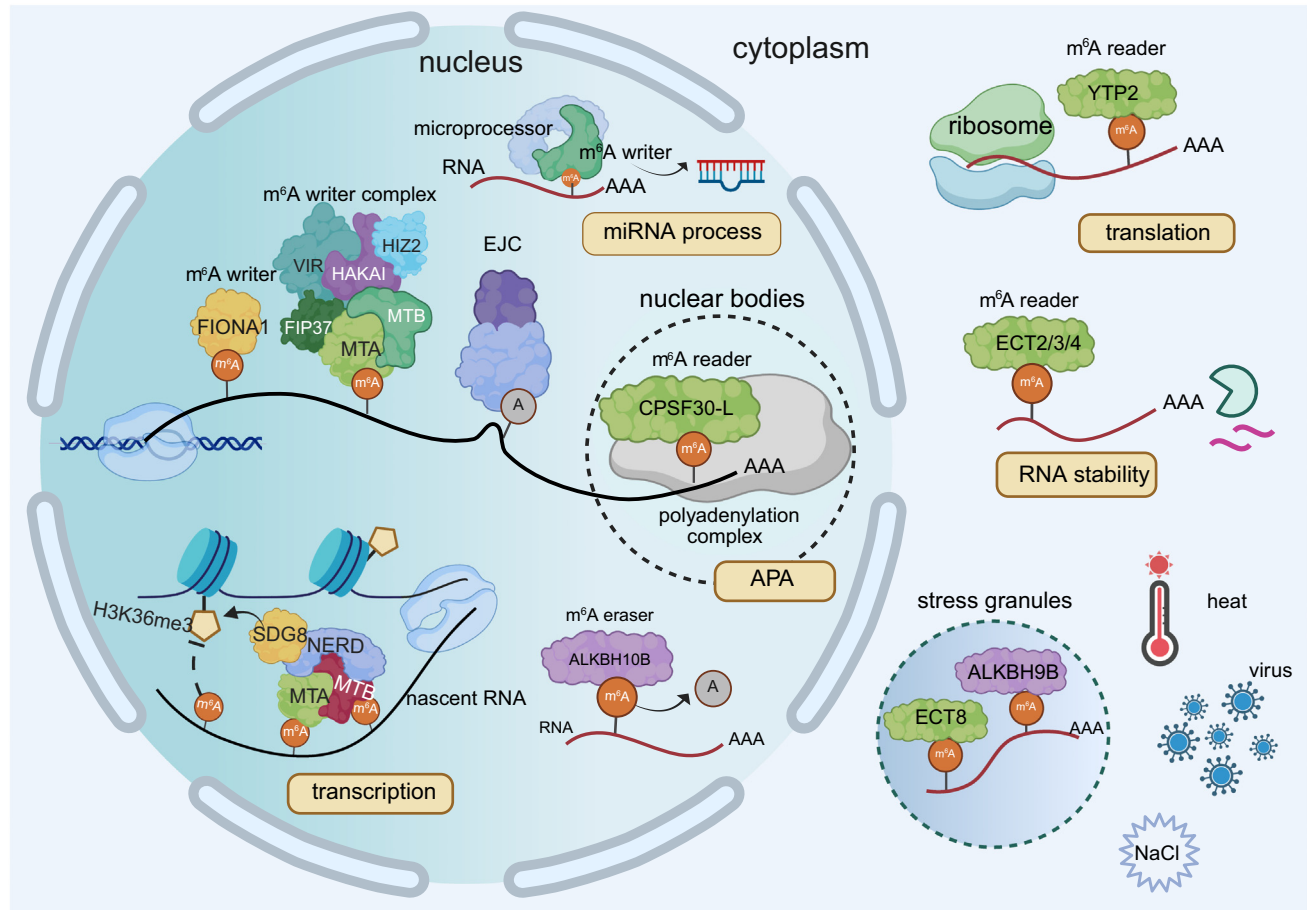
³China Sycamore Research Institute of Life Sciences, Shanghai 201203, China

⁴Fujian Agriculture and Forestry University, College of Life Science, Fuzhou 350002, China

⁵Basic Forestry and Proteomics Research Center, Fujian Provincial Key Laboratory of Haixia Plant Systems Biology, Haixia Institute of Science and Technology, Fujian Agriculture and Forestry University, Fuzhou 350002, China

[#]These authors contributed equally to this work

*Correspondence: lfgu@fafu.edu.cn (L. Gu).



Trends in Plant Science

Figure 1. m⁶A regulators in plants. m⁶A modification is catalyzed by the m⁶A methyltransferase complex, which contains MTA, MTB, FIP37, VIR, HAKAI, HIZ2, and NERD. FIONA1 is another m⁶A methyltransferase. m⁶A is removed by m⁶A demethylases ALKBH10B and ALKBH9B. Proteins containing YTH domains, such as ECT2, ECT3, ECT4, and CPSF30L, can recognize m⁶A modification. These regulators are critical for RNA metabolism, including transcription, polyadenylation, translation, RNA stability, and miRNA processing. The protein structures and cellular elements in this figure were created with [BioRender.com](https://www.biorender.com/). AAA: poly(A) tail; ALKBH10B: alpha-ketoglutarate-dependent dioxygenase homolog 10B; ALKBH9B: alpha-ketoglutarate-dependent dioxygenase homolog 9B; APA: alternative polyadenylation; CPSF30-L: CLEAVAGE AND POLYADENYLATION SPECIFICITY FACTOR30-L; ECT2: EVOLUTIONARILY CONSERVED C-TERMINAL REGION 2; EJC: exon junction complex; FIONA1: FIONA1 (FIO1), a plant homolog of METTL16; FIP37: FKBP12 interacting protein 37; H3K36me3: histone H3 lysine 36 trimethylation; HAKAI: E3 ubiquitin-protein ligase HAKAI; HIZ2: HAKAI-interacting zinc finger protein 2; m⁶A: N⁶-methyladenosine; MTA: mRNA adenosine methylase; MTB: methyltransferase B; NaCl, NERD: needed for RDR2-independent DNA methylation; SDG8: set domain group 8; VIR: VIRILIZER; YTP2: YTH domain-containing RNA-binding protein 2.

m⁶A regulation in trees is more complex, likely due to the expansion and diversification of m⁶A regulatory components. Many tree species have undergone whole-genome duplications and polyploidy events, including triploid ‘Yinzong’ poplar [10], tetraploid Moso bamboo [11], and hexaploid Ma bamboo [12]. This implies that trees may have an expanded regulatory network. For example, 14 putative writers, 14 putative erasers, and 33 putative readers have been identified in poplar 84K (*Populus alba* × *Populus tremula* var. *glandulosa*) [13], a total that exceeds the number of corresponding members in arabidopsis. Such expansion may allow duplicated genes to gain divergent functions or expression patterns, possibly playing critical roles in tree-specific processes like secondary growth and long-term stress adaptation. However, the identification of most m⁶A regulatory components in trees relies primarily on homologous sequence comparisons and phylogenetic analysis, and functional validation of these candidate genes could be prioritized in future research.

m⁶A writer complex in arabidopsis consists of the catalytic subunits MTA (mRNA adenosine methylase, ortholog of METTL3 (methyltransferase-like 3)) and MTB (mRNA adenosine methylase, ortholog of METTL14 (methyltransferase-like 14)), together with auxiliary subunits, including FIP37 (FKBP12 interacting protein 37, ortholog of WTAP (Wilms tumor 1 associated protein)), VIR (VIRILIZER, ortholog of VIRMA (vir-like m⁶A methyltransferase associated)), HAKAI (E3 ubiquitin-protein ligase HAKAI) [14–16], and NEEDED FOR RDR2 (RNA-dependent RNA polymerase 2)-INDEPENDENT DNA METHYLATION (NERD) (Figure 1) [17]. The MTA–MTB catalytic core is stabilized by FIP37 and VIR, and VIR promotes the accumulation of FIP37 and HAKAI in arabidopsis [18]. MTA can also deposit m⁶A on pri-miRNAs to modulate miRNA biogenesis [19]. NERD facilitates m⁶A deposition on nascent RNA by interacting with MTA and MTB [17]. Another m⁶A writer, FIONA1 (ortholog of METTL16 (methyltransferase-like 16)), primarily modifies coding regions of RNA (Figure 1) [20,21]. In contrast to model plants, studies on m⁶A writers in trees are relatively limited. Although homologs of most m⁶A writers have been identified in tree species, functional validation has mainly focused on a limited number of them. For example, overexpression of *PtrMTA* in poplar increases global m⁶A levels [22]. MdMTA and PpMTA70-4 function as m⁶A writers in apple and pear, respectively [23,24]. LkMTA2 and LkMTB8 form a heterodimer and work together to catalyze m⁶A modifications in larch [25]. PagFIP37, a component of m⁶A writer complex, was shown to positively regulate m⁶A deposition in poplar [5]. In apple, RNAi of m⁶A writer complex components *MdVIR1* and *MdVIR2* reduces global m⁶A levels, whereas their overexpression increases m⁶A deposition [26]. A recent study on poplar showed that PagMTC (a homolog of METTL4) can act as an m⁶A writer to enhance salt stress tolerance [27].

The biological functions of m⁶A modification are primarily mediated by reader proteins. In arabidopsis, 13 YTH (YT521-B homology) domain-containing proteins have been identified, and most of them belong to the EVOLUTIONARILY CONSERVED C-TERMINAL REGION (ECT) family [28]. The conserved YTH domain, characterized by consecutive tryptophan residues, is responsible for m⁶A binding. To date, a growing number of m⁶A readers have been identified in arabidopsis (Figure 1), including ECT2, ECT3, ECT4, ECT8 [2,29–35], and the recently reported ECT10 [32]. As the homolog of YTHCDC1 (YTH domain-containing protein 1), CLEAVAGE AND POLYADENYLATION SPECIFICITY FACTOR30-L (CPSF30-L) also functions as an m⁶A reader and regulates alternative polyadenylation in the nucleus [36]. There may be functional conservation of m⁶A readers between trees and model plants. For example, MhYTP2 (homolog of AtECT2) in apple and PpYTHDFE1 (homolog of AtECT8) from peach have been identified as m⁶A readers and exhibit binding specificity to m⁶A-modified RNA [37] (Figure 1).

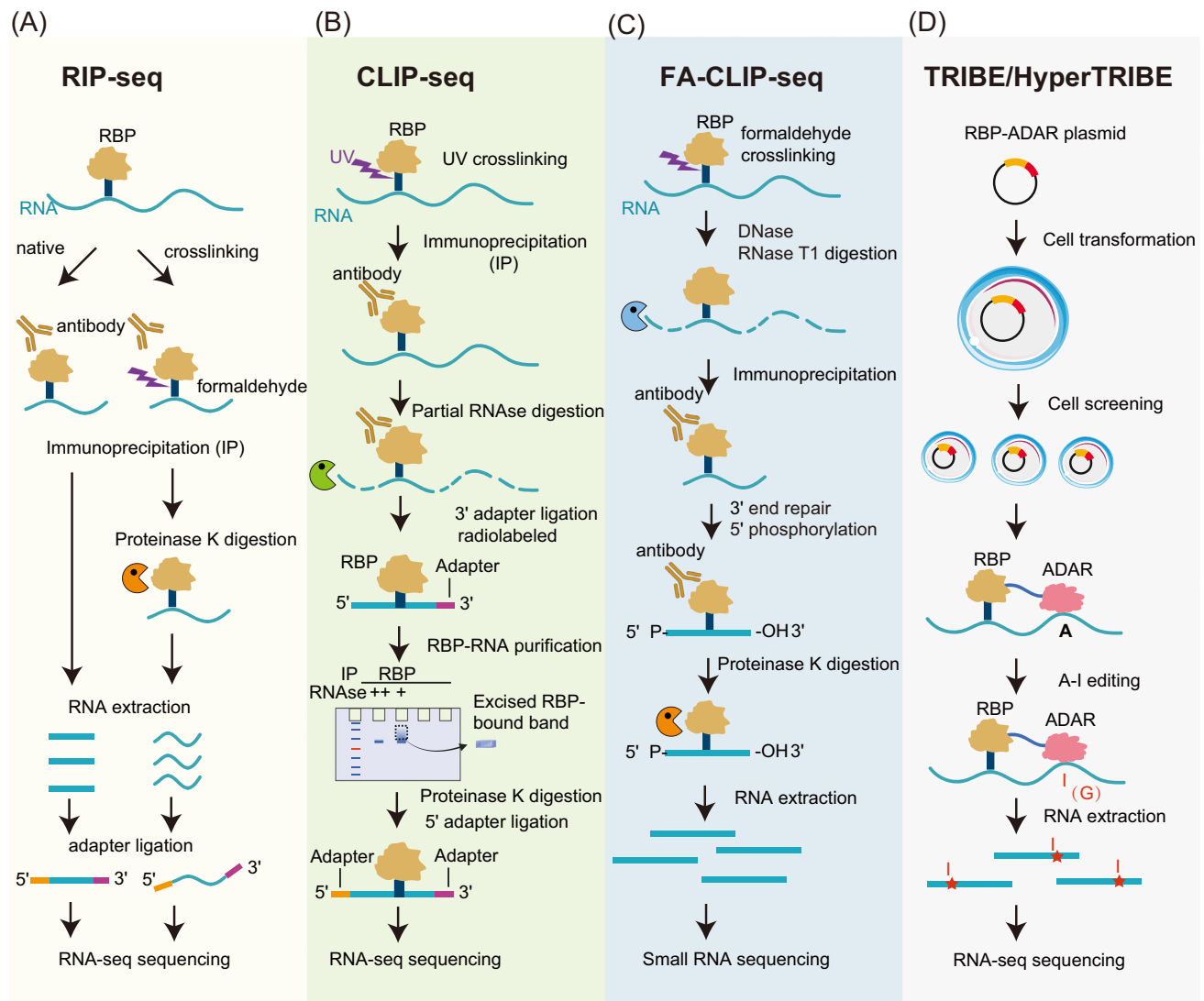
The m⁶A eraser ALKBH5 (alkB homolog 5) in mammals contains a conserved double-stranded β -helix (DS β H) fold and nucleotide recognition lid (NRL) region, which are crucial for RNA substrate binding [38]. In arabidopsis, six ALKBH (AlkB homolog) family members are homologous to ALKBH5, including ALKBH9A, ALKBH9B, ALKBH9C, ALKBH10A, ALKBH10B, and ALKBH10C [7]. However, sequence homology does not necessarily indicate conserved demethylase activity. To date, AtALKBH9B and AtALKBH10B in arabidopsis [3,39,40], as well as OsALKBH9 in rice [41,42], have been experimentally validated as m⁶A demethylases (Figure 1). In trees, PagALKBH9B, PagALKBH10B [43], and the recently identified PagALKBH1A [44] in poplar, PheALKBH9 in Moso bamboo [45], MdALKBH1A [46] from apple, and CsALKBH8 from citrus [47] have been demonstrated to possess m⁶A demethylase activity [45], whereas the biological functions of most ALKBH homologs remain largely unexplored.

Identification of target RNAs bound by m⁶A regulatory components

Identifying target RNAs is crucial for understanding the mechanisms of m⁶A regulatory components. The most widely used methods for mapping RNA–protein interactions include RNA

immunoprecipitation (RIP), ultraviolet crosslinking and immunoprecipitation (CLIP), formaldehyde crosslinking and immunoprecipitation (FA-CLIP), as well as editing-based approaches, such as TRIBE/hyperTRIBE (targets of RNA-binding proteins identified by editing) [48–51]. However, applying these approaches to tree species remains challenging due to low crosslinking efficiency or limited genetic transformation efficiency. The following section describes these approaches (Figure 2), discussing their underlying principles, advantages, and limitations in research on trees.

RIP enriches RNA-binding protein (RBP)–RNA complexes using specific antibodies. Proteinase K-digested RNA was collected for high-throughput sequencing to identify potential targets (Figure 2A, RIP-seq) [48,52,53]. Formaldehyde crosslinking strengthens RBP–RNA interaction [54]. Although



Trends in Plant Science

Figure 2. Methods for identifying RNA targets of RBPs. The diagram represents the principles of these methods: RNA immunoprecipitation (RIP-seq) provides a strategy for identifying RBP-associated RNAs, but depends on antibody quality (A). UV crosslinking immunoprecipitation (CLIP-seq) and formaldehyde crosslinking immunoprecipitation (FA-CLIP) improve interaction specificity through crosslinking, although their application is challenging in tree tissues (B, C). TRIBE/HyperTRIBE enables target identification without immunoprecipitation and is useful for tree species that are difficult to stably transform (D). ADAR: adenosine deaminase acting on RNA; HyperTRIBE: hyper targets of RNA-binding proteins identified by editing; RBP: RNA-binding protein; TRIBE: targets of RNA-binding proteins identified by editing.

RIP does not achieve single-base resolution, its advantages, such as simple operation and low cost, have enabled successful application in multiple plants. In tomato, RIP is used to demonstrate the binding of SIALKBH2 to the transcript of *SIDML2* (*DEMETETER-like DNA demethylase 2*) [55]. RIP assays show that phosphorylation of FIP37 enhances its binding ability to target RNAs in arabidopsis [56].

CLIP uses UV light to covalently crosslink RBPs with their target RNAs *in vivo*. After immunoprecipitation with specific antibodies, partial RNase digestion, and rigorous washing, RNA fragments are released by proteinase K and sequenced (Figure 2B) [50]. Several CLIP derivatives have been developed: HITS-CLIP (high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation) enables transcriptome-wide binding mapping [57]; PAR-CLIP (photoactivatable-ribonucleoside-enhanced crosslinking and immunoprecipitation) uses 4-thiouridine to enhance crosslinking efficiency [58]; iCLIP (individual-nucleotide resolution CLIP) achieves single-nucleotide resolution [59]; and eCLIP (enhanced CLIP)/sCLIP (simplified CLIP)/irCLIP (infrared CLIP) simplify the workflow [60–62].

FA-CLIP is an efficient approach that integrates FA-RIP and CLIP to identify RBP binding sites (Figure 2C) [35]. FA-CLIP utilizes formaldehyde to covalently crosslink protein–RNA interactions. After immunoprecipitation, protein–RNA complexes undergo partial RNA digestion, reverse transcription (generating mutation-containing cDNA), and Proteinase K digestion before library construction and high-throughput sequencing. In arabidopsis, this method has been applied to map transcriptome-wide binding sites of ECT2 [35] and CPSF30-L [36].

However, these antibody-dependent methods, including RIP-seq, CLIP-seq, and FA-CLIP, have several limitations in trees. These approaches generally rely on highly specific antibodies or genetic transformation to express epitope-tagged proteins for immunoprecipitation. The requirement for a large amount of material also restricts their application in scarce tissues such as the vascular cambium, secondary xylem, and buds. In addition, crosslinking efficiency is reduced in several tissues because of thick cell walls, high lignin content, or waxy leaf surfaces. Therefore, applying these methods to trees still requires extensive optimization of various aspects, such as crosslinking conditions, treatment time, and vacuum impregnation.

TRIBE identifies target RNAs by expressing a fusion protein of an RBP and the catalytic domain of the *Drosophila* RNA-editing enzyme ADAR (ADARcd) (Figure 2D). The fusion protein deaminates adenosine (A) to inosine (I) near the binding sites of target RNAs, which is recognized as guanosine (G) during sequencing, enabling identification of target RNAs [51]. To overcome limitations like low editing efficiency and sequence preferences, HyperTRIBE was developed by integrating the highly active mutant E488Q into ADARcd, thereby greatly improving sensitivity [63]. Although TRIBE/HyperTRIBE can only identify the target RNAs, rather than the precise binding sites, it provides a simple, low-cost, and effective method to study m⁶A-related proteins in trees. In arabidopsis, the HyperTRIBE method revealed substantial overlap between RNAs bound by ECT2 and those bound by ECT3 [30]. Moreover, HyperTRIBE has been employed to identify the target RNAs of PheMTA1 and PheMTA2 [64] and, more recently, to reveal the target RNAs of the m⁶A demethylase PheALKBH9 [45] in Moso bamboo via protoplast transformation.

A comparative overview of the key advantages and limitations of these methods is provided in Table S1 in the Supplemental information online. The choice of strategy depends on sample characteristics. RIP can identify candidate RNA targets but lacks resolution for precise binding sites. CLIP and FA-CLIP provide higher-resolution mapping of RNA–protein interactions, but their application in tree species is often limited by the availability of high-quality antibodies and by low crosslinking efficiency. In contrast, HyperTRIBE offers several advantages for studying tree

species because it does not require immunoprecipitation or UV crosslinking, making it suitable for lignified tissues, which are difficult to process. Furthermore, when used with tissue-specific promoters, HyperTRIBE has the potential to identify RNA targets in defined tissues, such as the vascular cambium. Importantly, HyperTRIBE allows rapid RNA target identification via transient expression methods, such as protoplast transformation. These features make HyperTRIBE a particularly valuable tool for tree species with long life cycles and low genetic transformation efficiency. Nevertheless, HyperTRIBE requires an efficient transient expression system and does not provide precise binding sites. Therefore, the selection of a strategy should be determined by balancing resolution, sample availability, and transformation feasibility.

m⁶A modification affects plant growth and development in model plants and trees

The involvement of m⁶A modification in growth and development has been reported in model plants and several tree species, including *Arabidopsis thaliana*, *Populus trichocarpa*, *Phyllostachys edulis*, *Malus domestica*, *Larix kaempferi*, and *Catalpa fargesii* (Figure 3) (Table S2 in the Supplemental information online). In *Arabidopsis*, inactivation of MTA or FIP37 results in embryo death and premature arrest of seed development, respectively [14,16], while FIP37 also controls shoot apical meristem proliferation (Figure 3A) [15]. Flowering is also modulated by m⁶A modification. FIONA1 prevents premature flowering by regulating the mRNA stability of flowering regulators [20,21]; NERD regulates flowering by promoting nascent

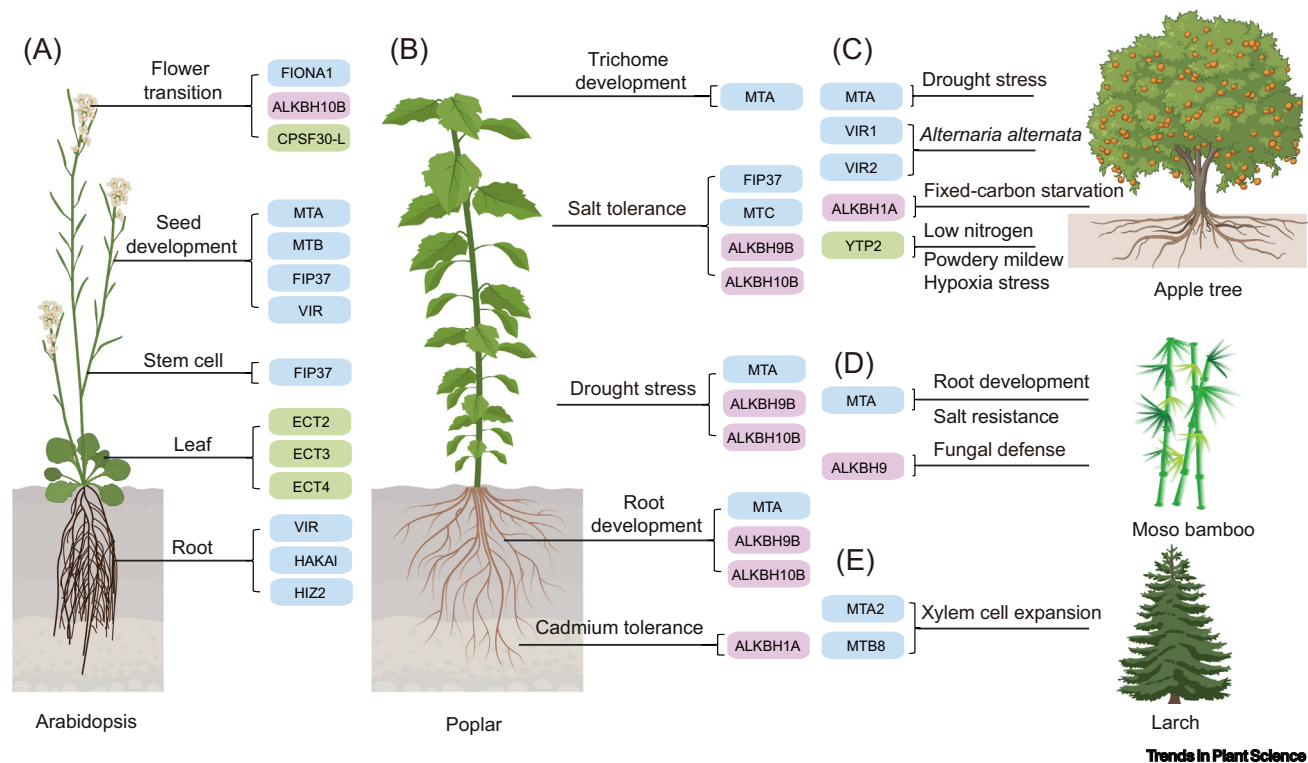


Figure 3. Biological functions of m⁶A regulatory components in Arabidopsis and major tree species. The figure shows the functions of m⁶A regulatory components in Arabidopsis (A), poplar (B), apple (C), Moso bamboo (D), and larch (E) from left to right. The boxes with blue, purple, and green backgrounds represent m⁶A writers, erasers, and readers, respectively. The diagrams of Arabidopsis, poplar, apple, and larch in this figure were created using BioRender.com. ALKBH1A: AlkB homolog 1A; ALKBH9: AlkB homolog 9; ALKBH9B: AlkB homolog 9B; ALKBH10B: AlkB homolog 10B; CPSF30-L: CLEAVAGE AND POLYADENYLATION SPECIFICITY FACTOR30-L; ECT2: EVOLUTIONARILY CONSERVED C-TERMINAL REGION 2; FIONA1: (FIO1), a plant homolog of METTL16; FIP37: FKBP12 interacting protein 37; HAKAI: E3 ubiquitin-protein ligase HAKAI; HIZ2: HAKAI-interacting zinc finger protein 2; m⁶A: N⁶-methyladenosine; MTA: mRNA adenosine methylase; MTB: methyltransferase B; MTC: a plant homolog of methyltransferase-like 4 (METTL4); VIR: VIRILIZER, ortholog of VIRMA; YTP2: YTH domain-containing protein 2.

RNA m⁶A deposition and suppressing H3K36me3 histone modification at *FLC* [17]; and ALKBH10B influences the flowering transition by modulating m⁶A-dependent mRNA stability [3,40]. Beyond flowering, ECT2/3/ECT4 redundantly control the timing of organ initiation and leaf morphology [2,29]. The putative m⁶A demethylase AtALKBH9C contributes to seed germination and seedling growth [65].

In contrast, studies on forest trees have revealed that m⁶A plays unique roles in root development and wood formation. In poplar, overexpression of *PtrMTA* significantly enhances trichome density and promotes root development (Figure 3B) [22]. The m⁶A demethylases PagALKBH9B and PagALKBH10B also play a critical role in adventitious root formation and biomass accumulation [43]. In apple, knockdown of *MdMTA* causes impaired root development and reduced plant height (Figure 3C) [23]. In Moso bamboo, under treatment with an RNA methylation inhibitor, the number of lateral roots increases, and the m⁶A level on the 3'-UTR (Untranslated region) of transcripts is significantly reduced, accompanied by increased gene expression [66]. Recent research demonstrates that *PheMTA* from Moso bamboo significantly enhances root elongation in plants (Figure 3D) [64]. In larch, the LkMTA2/LkMTB8 methyltransferase complex regulates the expansion of xylem cells through the *Lkerf1* (*ETHYLENE-RESPONSIVE FACTOR 1*)—*Lkexpa8* (*EXPANSIN A8*) cascade, thereby playing a role in wood formation (Figure 3E) [25].

During the growth of Moso bamboo from 2 to 4 m in height, m⁶A modifications tend to be gradually demethylated, with lignin-related transcripts exhibiting lower m⁶A levels alongside increased transcript abundance [67]. In addition to coding genes, m⁶A modifications on circular RNAs in Moso bamboo seedlings have been identified [68]. In poplar, an analysis of 61 m⁶A pathway-like genes showed tissue-specific expression in leaves, xylem, phloem, and roots [13]. The silencing of *CfALKBH5*, a putative m⁶A demethylase, caused a chlorotic phenotype in *Catalpa fargesii* [69].

m⁶A modification alters the resistance of plants to abiotic stress

In arabidopsis, m⁶A regulators modulate cold, salt, and drought stress responses. Knockdown of *MTA* reduces the translation efficiency of m⁶A-modified cold-responsive genes, causing abnormal growth in arabidopsis under cold stress [70]. Mutations in *MTA*, *MTB*, *VIR*, *HAKAI*, or *FIONA1* cause salt sensitivity by altering m⁶A modification [71,72]. The m⁶A reader ECT8 accelerates the degradation of target m⁶A-modified RNAs in processing bodies, which is vital for salt tolerance (Figure 1) [31]. ECT10 negatively regulates the drought response by affecting mRNA stability [32]. Knockout of *ALKBH9B*, the m⁶A eraser, increases sensitivity to ABA treatment [31], and ALKBH10B influences drought tolerance by modulating mRNA stability [3,40]. The putative demethylase AtALKBH9C is also involved in abiotic stress responses [65].

In tree species, emerging evidence reveals the conserved role of m⁶A modification in stress resistance, and the perennial nature of trees might further highlight its key role in long-term environmental adaptation. In poplar, multiple m⁶A writer components, including *PtrMTA* [22], *PagFIP37* [5], and *PagMTC* [27], improve stress tolerance by regulating the stability of critical stress-response transcripts (Figure 3B). Overexpression of *PheMTA* from Moso bamboo significantly enhances salt-stress tolerance in rice (Figure 3D) [64]. In apple, *MdMTA*-mediated m⁶A modification enhances drought resistance by promoting lignin deposition and scavenging excessive H₂O₂ [23] (Figure 3C). *MhYTP2* in apple participates in regulating tolerance to low-nitrogen [73]. m⁶A erasers in trees also mediate key abiotic stress responses. For instance, PagALKBH9B and PagALKBH10B enhance salt tolerance by reducing H₂O₂ accumulation and oxidative damage in poplar [43]. Overexpression of *PheALKBH9* from Moso bamboo makes rice more sensitive to salt stress (Figure 3D) [45]. PagALKBH1A-mediated m⁶A demethylation negatively regulates cadmium stress tolerance in 84K poplar [44].

MdALKBH1A reduces high-temperature resistance but increases fixed-carbon starvation resistance in apple [46].

m⁶A modification is involved in the response to biotic stress in plants

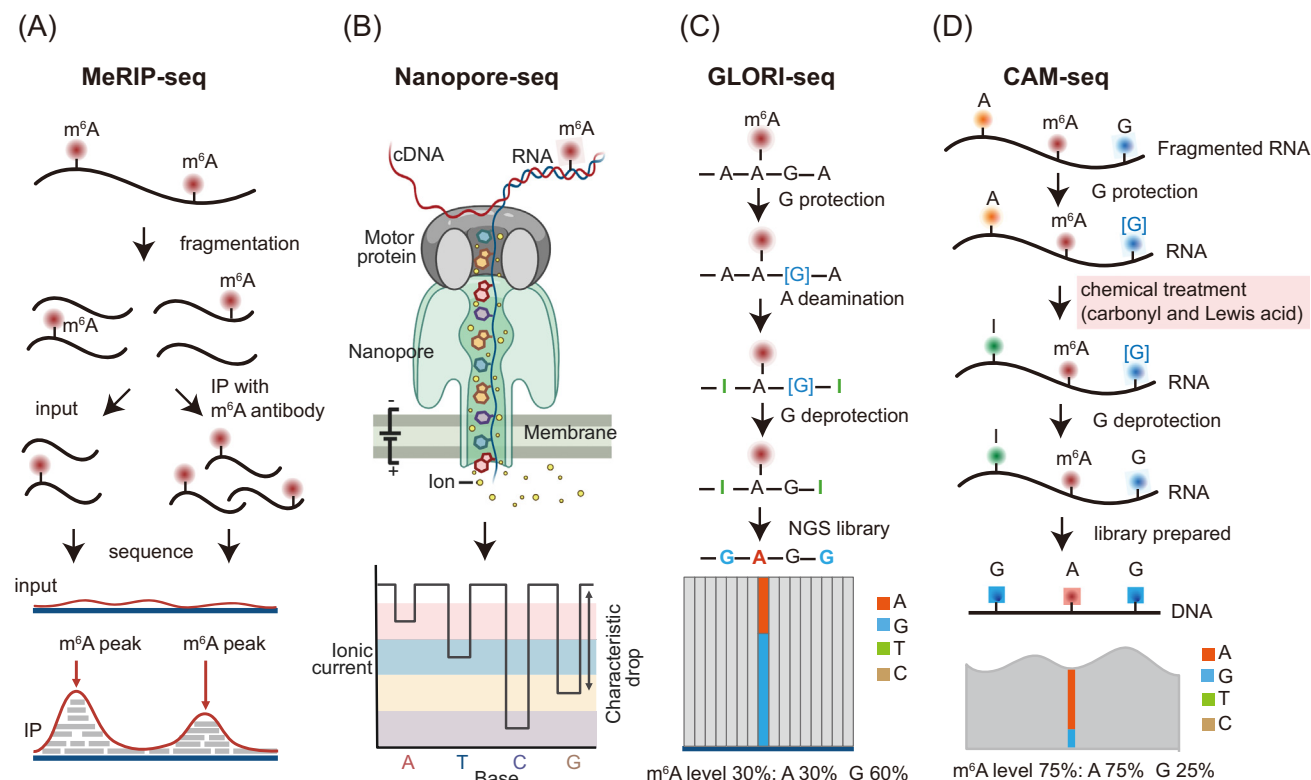
The impact of m⁶A modification on plant–pathogen interactions is highly complex. Overexpression of NbMETTL1 (methyltransferase-like protein 1) and NbMETTL2 (methyltransferase-like protein 2) enhanced plum pox virus (PPV) resistance in *Nicotiana benthamiana* [74], while downregulation of NbALKB1 (AlkB homolog 1) and NbALKB2 (AlkB homolog 2), two members of the ALKBH9 clade, reduced PPV and potato virus Y accumulation [75]. Similarly, the m⁶A methyltransferase TaMTB in *Triticum aestivum* acts as a positive regulator of WYMV (wheat yellow mosaic virus) infection [76]. In arabidopsis, a mutation in the m⁶A demethylase *AtALKBH9B* leads to reduced alfalfa mosaic virus (AMV) accumulation, suggesting that *AtALKBH9B* may positively regulate AMV infection (Figure 1) [77]. Studies in trees also demonstrate that m⁶A modification influences pathogen infection. Overexpression of *PpMTA70-4* significantly enhances pear resistance to *Penicillium expansum* infection [24]. In apple, m⁶A modification mediated by MdVIR1 and MdVIR2 improves mRNA stability and translation efficiency of *MdWRKY79* (*WRKY transcription factor 79*) and *MdNLR16* (*nucleotide-binding leucine-rich repeat protein 16*) and *MdNLR16*, thereby increasing resistance to *Alternaria alternata* [26]. m⁶A reader MhYTP2 degrades the m⁶A-modified RNAs of *MdMLO19* (*Mildew Locus O 19*) and *MdMLO19-X1* (*Mildew Locus O 19, isoform X1*) and improves the translation efficiency of antioxidant genes, thereby enhancing resistance to powdery mildew in apple [37]. PheALKBH9, an m⁶A demethylase in Moso bamboo, influences defense responses to fungal pathogens via its demethylation activity (Figure 3D) [45]. The citrus m⁶A demethylase CsALKBH8 contributes to postharvest resistance against *Penicillium digitatum* [47].

Strategies for m⁶A modification detection

The development of m⁶A detection methods has significantly promoted exploration in m⁶A research. Thin-layer chromatography [14,78], dot blot [79], and liquid chromatography–tandem mass spectrometry [80] are widely employed to assess global m⁶A modification levels in organisms. However, these strategies are unable to reveal the precise sites of m⁶A modification on transcripts. In the following section, we summarize the methods commonly used to identify m⁶A sites (Figure 4), including antibody-based methods like MeRIP-seq (methylated RNA immunoprecipitation sequencing)/m⁶A-seq [81,82] and m⁶A-CLIP [83,84], and antibody-independent sequencing, such as Nanopore direct RNA sequencing (DRS) [85,86], GLORI-seq (glyoxal and nitrite-mediated deamination of unmethylated adenosines) [87], m⁶A-SAC-seq (selective allyl chemical labeling and sequencing) [88], and CAM-seq (chemical cooperative catalysis-assisted N⁶-methyladenosine sequencing) [89].

MeRIP-seq/m⁶A-seq, the first antibody-based m⁶A profiling method, uses fragmented mRNA (100–200 nt) that is immunoprecipitated with an m⁶A antibody, followed by sequencing to identify methylation peaks (Figure 4A) [81,82]. Although the resolution is only about 200 nt, its simplicity and robust pipelines make it widely used in model plant and tree species, revealing m⁶A dynamics during salt stress in hybrid poplar [27]. The upgraded version, m⁶A-seq2, enables barcode-based pooling of multiple samples, reducing the cost and time required for comparative analyses [85]. For single-base resolution, m⁶A crosslinking and immunoprecipitation (m⁶A-CLIP)/miCLIP uses UV crosslinking and reverse transcription to introduce mutations near the m⁶A site, allowing precise localization, though it may struggle with adjacent adenines [83,84].

To overcome the limitations of dependence on antibodies, antibody-independent m⁶A sequencing methods such as DRS have been developed. DRS can reveal m⁶A modifications [85,86] based on the signal difference between the modified base and the canonical base as mRNA passes through



Trends in Plant Science

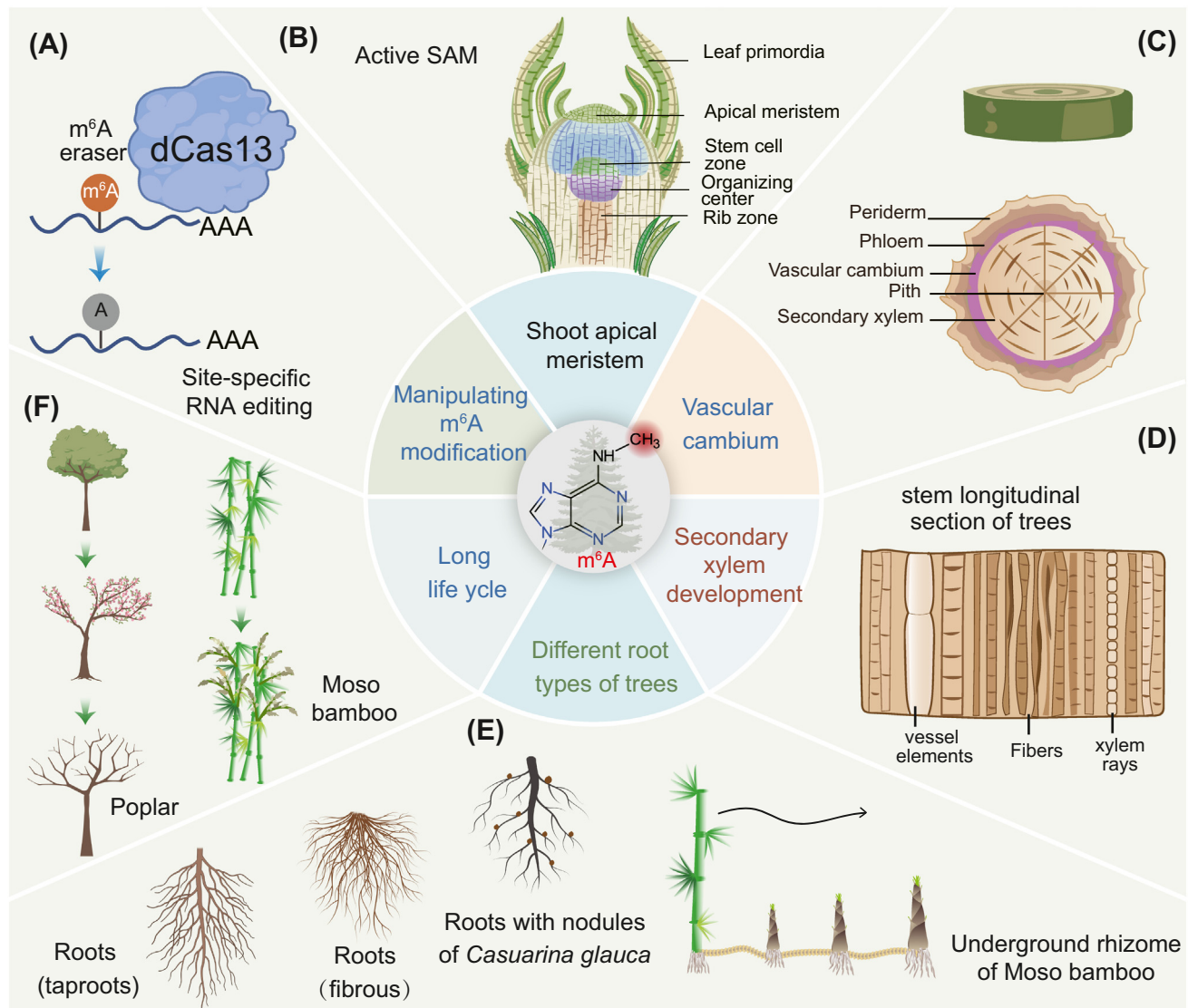
Figure 4. Technologies to identify m⁶A sites in plants. MeRIP-seq enables transcriptome-wide m⁶A profiling but provides limited resolution (A). Direct RNA sequencing provides information on various modifications of full-length transcripts and isoforms, offering advantages for complex analyses in tree species (B). GLORI and CAM-seq offer mild reaction conditions, high specificity, and low background noise (C, D). The nanopore schematic in this figure was created with [BioRender.com](https://www.biorender.com). MeRIP-seq: methylated RNA immunoprecipitation sequencing; DRS: nanopore direct RNA sequencing; GLORIseq: glyoxal and nitrite-mediated deamination of unmethylated adenosines sequencing; CAM-seq: chemical cooperative catalysis-assisted N⁶-methyladenosine sequencing.

the nanopores (Figure 4B). Multiple algorithms, such as Tombo, EpiNano, XPore, Nanocompare, and Nanom6A, have been developed to analyze DRS data with high reliability [86,90,91]. For example, m⁶A sites predicted by DRS show concordance with those identified by MeRIP-seq in poplar [86]. This method has also been applied to Moso bamboo [66]. Although DRS enables single-base detection of m⁶A without reverse transcription or PCR, its application remains constrained by high costs, low sequencing depth, and substantial RNA input requirements.

GLORI achieves single-base resolution by deaminating unmethylated adenosine (A) to inosine (I) via glyoxal and nitrite, while m⁶A remains unchanged; during reverse transcription, I is read as G (Figure 4C) [87]. Despite its precision, the complex reaction risks RNA degradation, limiting low-input applications. Optimized versions address this problem: GLORI 2.0 uses a one-pot deamination that reduces reaction time from hours to 10 min and preserves RNA integrity, enabling m⁶A quantification from approximately 10,000 cells. GLORI 3.0 further enhances sensitivity for ultra-low inputs (~500–1000 cells) by incorporating carrier RNA [92]. Together, these iterations make GLORI a powerful tool for scarce samples.

m⁶A-SAC-seq offers another quantitative, single-base-resolution method for low-input samples [~30 ng of poly(A) or rRNA-depleted RNA]. It utilizes MjDim1 to convert m⁶A into a⁶m⁶A, using allylic-SAM (S-adenosylmethionine) as a cofactor, followed by cyclization under I₂ treatment. The

cyclized m^6A generates mutations during reverse transcription, enabling precise identification [88]. CAM-seq is the latest method (Figure 4D), using cooperative catalysis (a carbonyl organocatalyst and a Lewis acid) for mild deamination of unmethylated adenine (read as G), while m^6A remains A [89]. It enables fast, cost-effective m^6A profiling for low-input samples, detecting approximately 200 000 m^6A sites from 10 ng of mRNA with 0.5% background noise. Other quantification methods include MAZTER-seq (RNA digestion via m^6A sensitive RNase) and m^6A -REF-seq (m^6A -sensitive RNA-endoribonuclease-facilitated sequencing) [93,94], m^6A -SEAL-seq (m^6A selective chemical labeling sequencing) [95], and eTAM-seq (evolved TadA-assisted N^6 -methyladenosine sequencing) [96], collectively expanding the strategies for m^6A detection.



Trends in Plant Science

Figure 5. Future directions of m^6A research in trees. Given the perennial life history of trees, key areas for future m^6A research include targeted manipulation of m^6A using epitranscriptomic editors (A); shoot apical meristem (SAM) activity and dormancy (B); vascular cambium activity and secondary xylem formation (C, D); root diversity (E); and reproductive development in long-lived trees (F). The poplar schematic (F) and the diagrams of the three roots on the left (E) were created with BioRender.com. AAA: poly(A) tail; dCas13: catalytically active Cas13; m^6A : N^6 -methyladenosine.

The selection of an appropriate m⁶A detection method depends on factors such as resolution, quantitative capability, and RNA sample requirements (Table S3 in the Supplemental information online). For transcriptome-wide mapping, MeRIP-seq/m⁶A-seq remains the most established method, albeit with limited resolution. Single-base precision can be achieved with GLORI-seq, m⁶A-SAC-seq, and CAM-seq, which are suitable for low-input RNA or single-cell applications. Nanopore DRS provides more comprehensive information, including identification of multiple modification types at single-transcript resolution, which makes it the most flexible option, although it requires high costs and complex bioinformatics analyses. Current m⁶A profiling in trees relies primarily on MeRIP-seq and Nanopore DRS [27,66,86]. GLORI-seq, m⁶A-SAC-seq, and CAM-seq have yet to be applied in trees. They could be widely applied to tree species. These approaches may provide improved resolution and quantitative information for m⁶A profiling in trees.

How can m⁶A modifications be precisely manipulated using epigenetic editors?

Current understanding of m⁶A functionality primarily relies on phenotypic observations resulting from overexpressing or mutating m⁶A regulatory components to infer potential biological roles. However, perturbation of these regulators can alter m⁶A levels at numerous sites across the transcriptome. To move beyond these systemic approaches, deciphering the function of m⁶A at single-base resolution is crucial. For m⁶A sites in 3'UTRs, genome editing of the modified adenosine can directly link methylation to biological phenotypes [97]. Prime editing achieves exact nucleotide substitutions, providing a powerful method to modify specific m⁶A motifs [98,99]. However, this strategy is less suitable for m⁶A sites located in CDS (Coding sequence) regions because nucleotide substitutions may alter the amino acid sequence or codon usage preferences, making it more complicated to determine the function of m⁶A. Emerging methods utilizing dCas13 (catalytically dead Cas13 protein)-fused RNA methyltransferases or demethylases enable targeted installation or removal of specific m⁶A modifications without changing the RNA or DNA sequence (Figure 5A) [100–102]. We anticipate that such approaches will increasingly unravel the functional significance of individual m⁶A sites and facilitate the development of precision breeding in tree species, including secondary growth, lignin formation, and resistance to stresses. For example, manipulation of m⁶A sites in transcripts associated with hormone signaling and root development may enhance growth rate. Similarly, modifying m⁶A on genes involved in secondary xylem and lignin accumulation could provide new strategies for optimizing wood quality. In addition, precise regulation of m⁶A on stress response transcripts may enhance tolerance to drought, salt, extreme temperatures, and pathogen infections, which is particularly important for long-lived tree species.

However, applying the approach to tree species remains challenging. Lower genetic transformation efficiency and longer regeneration times make it difficult to stably express the dCas13 fusion protein in trees. Transient expression platforms, including protoplast transformation and virus-mediated delivery systems, provide opportunities for rapid functional validation without requiring stable genetic transformation. Nevertheless, current transient expression methods in tree species remain constrained by low delivery efficiency, restricted host range, and limited packaging capacity of viral vectors. Therefore, improving delivery efficiency, expanding transient expression systems, and developing compact programmable RNA editors are essential for elucidating the functions of individual m⁶A sites in trees.

Concluding remarks and future perspectives

Although m⁶A research in tree species remains at an early stage, its significance is increasingly being recognized. Accumulating evidence demonstrates its critical role in wood formation, root development, and stress responses, which is particularly important for long-lived trees. For instance, the latest report on larch shows that the LkMTA2/LkMTB8 methyltransferase complex promotes xylem cell expansion by enhancing mRNA stability and the abundance of LkERF1, revealing the key role of m⁶A modification in wood formation [25]. PtrMTA (poplar), PheMTA (Moso bamboo),

Outstanding questions

Compared with herbaceous model plants, have m⁶A writers, readers, and erasers undergone functional diversification in woody perennials?

Does m⁶A modification directly regulate unique development of tree species, such as meristem activity and dormancy, secondary xylem growth, vascular differentiation, and lignin deposition?

How to explore m⁶A functions in tree species which are difficult to stably transform?

Can programmable RNA epigenetic editors enable causal dissection of individual m⁶A sites in trees?

and MdMTA (apple) promote root development [22,23,64], while PagFIP37 (poplar) and PheMTA improve salt tolerance [5,64]. Furthermore, the apple m⁶A reader MhYTP2 regulates resistance to biotic stress by altering RNA stability and the translation efficiency of target RNAs [37,73,103,104].

These findings represent an initial exploration of the role of m⁶A in trees; however, the contribution of m⁶A regulation to the growth and adaptation of perennial trees is still largely unresolved. Future research should focus on the unique biological characteristics of trees. First, researchers should reveal m⁶A dynamics in meristem activity and dormancy (Figure 5B). The shoot apical meristem (SAM) undergoes seasonal transitions between active growth and dormancy. m⁶A profiling of the SAM across seasons might reveal key mechanisms regulating perennial rhythms. Second, researchers should explore the m⁶A network in the vascular cambium and wood formation (Figure 5C,D). The cambium continuously produces secondary xylem and phloem, representing one of the most distinctive tissues in trees. It would be promising to identify m⁶A-regulated genes involved in cambial stem cell maintenance and secondary xylem development, thereby providing potential targets for improving wood properties. Third, researchers should uncover m⁶A mechanisms in root system diversity (Figure 5E). Root systems vary considerably among tree species. Poplars develop extensive lateral and adventitious roots, whereas Moso bamboo has a well-developed underground rhizome system, and nitrogen-fixing trees such as *Casuarina* form root nodules through symbiosis with *Frankia*. These specialized structures play key roles in nutrient support and transport, asexual reproduction, and stress adaptation. Future studies could investigate m⁶A dynamics during rhizome extension, nodule formation, and lateral root branching, revealing how m⁶A participates in root diversity.

Unlike the model plant *Arabidopsis*, many tree species require years or even decades to reach reproductive maturity (Figure 5F). Such long generation times complicate studies of the roles of m⁶A in reproductive development, particularly because obtaining T1 progeny and conducting genetic segregation analyses can be time-consuming. Although stable genetic transformation systems have been established in several trees, including poplar and apple, as well as in Moso bamboo, transformation and plant regeneration remain lengthy procedures, and efficient genetic tools are still lacking for many tree species. Therefore, transient approaches may provide useful alternatives. For example, the Bamboo mosaic virus-based system has been developed for transient gene expression and genome editing in Moso bamboo, allowing target genes to be examined without relying on stable transformation [105–107]. This system may provide an alternative strategy for programmable epigenetic editors to manipulate m⁶A modification. Heterologous expression of m⁶A regulatory components from trees in phylogenetically related species with shorter life cycles provides a practical and efficient strategy. For example, heterologous expression of key m⁶A regulators from Moso bamboo into rice provides insights into conserved mechanisms of m⁶A regulation among Poaceae species. However, this method is limited by the inherent differences in genetic background and regulatory networks between heterologous hosts and the original tree species. Therefore, more suitable strategies still need to be developed for the field (see also Outstanding questions).

Author contributions

L.G. led the project planning. H.H.W., H.Y.W., and L.G. wrote the manuscript and prepared the figures and tables. X.J., Y.W., L.Z., and B.J. revised the article. All authors read and approved the final version.

Acknowledgments

This research was funded by the National Key R&D Program of China (2023YFD2200201), the National Natural Science Foundation of China (32371980), the China Postdoctoral Science Foundation (2025M781858), the Natural Science Foundation of Fujian Province (2025J02014), the Fujian Provincial Forestry Science and Technology Project (2026FKJ5), the S&T Innovation (KFB23180 and KFB24096A), and the Forestry Peak Discipline Construction Project of Fujian Agriculture and Forestry University (725025010 and 72202200205).

Declaration of interests

The authors declare no competing interests.

Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tplants.2026.08.011>.

References

- Batista, P.J. *et al.* (2014) m⁶A RNA modification controls cell fate transition in mammalian embryonic stem cells. *Cell Stem Cell* 15, 707–719
- Arribas-Hernandez, L. *et al.* (2020) Recurrent requirement for the m⁶A-ECT2/ECT3/ECT4 axis in the control of cell proliferation during plant organogenesis. *Development* 147, dev189134
- Duan, H.-C. *et al.* (2017) ALKBH10B is an RNA N⁶-methyladenosine demethylase affecting *Arabidopsis* floral transition. *Plant Cell* 29, 2995–3011
- Yu, Q. *et al.* (2021) RNA demethylation increases the yield and biomass of rice and potato plants in field trials. *Nat. Biotechnol.* 39, 1581–1588
- Zhao, Y. *et al.* (2024) N⁶-methyladenosine mRNA methylation positively regulated the response of poplar to salt stress. *Plant Cell Environ.* 47, 1797–1812
- Yue, H. *et al.* (2019) N⁶-methyladenosine regulatory machinery in plants: composition, function and evolution. *Plant Biotechnol. J.* 17, 1194–1208
- Liang, Z. *et al.* (2020) Epigenetic modifications of mRNA and DNA in plants. *Mol. Plant* 13, 14–30
- Shao, Y. *et al.* (2021) N⁶-methyladenosine modification underlies messenger RNA metabolism and plant development. *Curr. Opin. Plant Biol.* 63, 102047
- Ramakrishnan, M. *et al.* (2024) Exploring N⁶-methyladenosine (m⁶A) modification in tree species: opportunities and challenges. *Hortic. Res.* 11, uhad284
- Chen, S. *et al.* (2023) Chromosome-level genome assembly of a triploid poplar *Populus alba* 'Berolinensis'. *Mol. Ecol. Resour.* 23, 1092–1107
- Zhao, H. *et al.* (2018) Chromosome-level reference genome and alternative splicing atlas of moso bamboo (*Phyllostachys edulis*). *Gigascience* 7, giy115
- Zheng, Y. *et al.* (2022) Allele-aware chromosome-scale assembly of the allopolyploid genome of hexaploid Ma bamboo (*Dendrocalamus latiflorus* Munro). *J. Integr. Plant Biol.* 64, 649–670
- Sun, X. *et al.* (2022) Genome-wide identification of m⁶A writers, erasers and readers in poplar 84K. *Genes (Basel)* 13, 1018
- Zhong, S. *et al.* (2008) MTA is an *Arabidopsis* messenger RNA adenosine methylase and interacts with a homolog of a sex-specific splicing factor. *Plant Cell* 20, 1278–1288
- Shen, L. *et al.* (2016) N⁶-methyladenosine RNA modification regulates shoot stem cell fate in *Arabidopsis*. *Dev. Cell* 38, 186–200
- Vespa, L. *et al.* (2004) The immunophilin-interacting protein AtFIP37 from *Arabidopsis* is essential for plant development and is involved in trichome endoreduplication. *Plant Physiol.* 134, 1283–1292
- Shao, Y. *et al.* (2025) NERD-dependent m⁶A modification of the nascent FLC transcript regulates flowering time in *Arabidopsis*. *Nat. Plants* 11, 468–482
- Shen, L. (2023) Functional interdependence of N⁶-methyladenosine methyltransferase complex subunits in *Arabidopsis*. *Plant Cell* 35, 1901–1916
- Bhat, S.S. *et al.* (2020) mRNA adenosine methylase (MTA) deposits m⁶A on pri-miRNAs to modulate miRNA biogenesis in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U. S. A.* 117, 21785–21795
- Wang, C. *et al.* (2022) FIONA1 is an RNA N⁶-methyladenosine methyltransferase affecting *Arabidopsis* photomorphogenesis and flowering. *Genome Biol.* 23, 40
- Xu, T. *et al.* (2022) FIONA1-mediated m⁶A modification regulates the floral transition in *Arabidopsis*. *Adv. Sci. (Weinh.)* 9, 2103628
- Lu, L. *et al.* (2020) MTA, an RNA m⁶A methyltransferase, enhances drought tolerance by regulating the development of trichomes and roots in poplar. *Int. J. Mol. Sci.* 21, 2462
- Hou, N. *et al.* (2022) MdMTA-mediated m⁶A modification enhances drought tolerance by promoting mRNA stability and translation efficiency of genes involved in lignin deposition and oxidative stress. *New Phytol.* 234, 1294–1314
- Xu, M. *et al.* (2026) The m⁶A methyltransferase subunit MTA70-4 confers resistance against *Penicillium expansum* in pear (*Pyrus pyrifolia* Nakai). *Postharvest Biol. Technol.* 242, 114603
- Lyu, Z. *et al.* (2026) The LkMTA2/LkMTB8-LkERF1-LkEXPA8 cascade regulates xylem cell expansion in larch. *New Phytol.* 250, 1535–1550
- Song, Z. *et al.* (2025) Nanopore RNA direct sequencing identifies that m⁶A modification is essential for sorbitol-controlled resistance to *Alternaria alternata* in apple. *Dev. Cell* 60, 1439–1453.e5
- Wang, R. *et al.* (2025) Dynamics of N⁶-methyladenosine (m⁶A) under salt stress in poplar and the intrinsic mechanism by which METTL4 (PagMTC) enhances salt tolerance. *Plant J.* 124, e70537
- Wang, N. *et al.* (2014) Genome-wide identification of members in the YTH domain-containing RNA-binding protein family in apple and expression analysis of their responsiveness to senescence and abiotic stresses. *Gene* 538, 292–305
- Arribas-Hernandez, L. *et al.* (2018) An m⁶A-YTH module controls developmental timing and morphogenesis in *Arabidopsis*. *Plant Cell* 30, 952–967
- Arribas-Hernandez, L. *et al.* (2021) The YTHDF proteins ECT2 and ECT3 bind largely overlapping target sets and influence target mRNA abundance, not alternative polyadenylation. *Elife* 10, e72377
- Cai, Z. *et al.* (2024) The m⁶A reader ECT8 is an abiotic stress sensor that accelerates mRNA decay in *Arabidopsis*. *Plant Cell* 36, 2908–2926
- Han, R. *et al.* (2025) ECT10 is an m⁶A reader that negatively regulates the drought stress response in *Arabidopsis* by modulating mRNA stability. *Plant J.* 124, e70637
- Meyer, K.D. and Jaffrey, S.R. (2017) Rethinking m⁶A readers, writers, and erasers. *Annu. Rev. Cell Dev. Biol.* 33, 319–342
- Scutenaire, J. *et al.* (2018) The YTH domain protein ECT2 is an m⁶A reader required for normal trichome branching in *Arabidopsis*. *Plant Cell* 30, 986–1005
- Wei, L.-H. *et al.* (2018) The m⁶A reader ECT2 controls trichome morphology by affecting mRNA stability in *Arabidopsis*. *Plant Cell* 30, 968–985
- Song, P. *et al.* (2021) *Arabidopsis* N⁶-methyladenosine reader CPSF30-L recognizes FUE signals to control polyadenylation site choice in liquid-like nuclear bodies. *Mol. Plant* 14, 571–587
- Guo, T. *et al.* (2022) The m⁶A reader MhYTP2 regulates MdMLO19 mRNA stability and antioxidant genes translation efficiency conferring powdery mildew resistance in apple. *Plant Biotechnol. J.* 20, 511–525
- Li, Q. and Zhu, Q. (2023) The role of demethylase AlkB homologs in cancer. *Front. Oncol.* 13, 1153463
- Tang, J. *et al.* (2022) The RNA N⁶-methyladenosine demethylase ALKBH9B modulates ABA responses in *Arabidopsis*. *J. Integr. Plant Biol.* 64, 2361–2373
- Han, R.P. *et al.* (2023) ALKBH10B-mediated m⁶A demethylation is crucial for drought tolerance by affecting mRNA stability in *Arabidopsis*. *Environ. Exp. Bot.* 209, 105306
- Xue, F. *et al.* (2024) m⁶A demethylase OsALKBH5 is required for double-strand break formation and repair by affecting mRNA stability in rice meiosis. *New Phytol.* 244, 2326–2342
- Tang, J. *et al.* (2024) OsALKBH9-mediated m⁶A demethylation regulates tapetal PCD and pollen exine accumulation in rice. *Plant Biotechnol. J.* 22, 2410–2423

43. Zhao, Y. *et al.* (2022) Genome-wide identification of the *AlkB* homologs gene family, *PagALKBH9B* and *PagALKBH10B* regulated salt stress response in *Populus*. *Front. Plant Sci.* 13, 994154
44. Wang, R. *et al.* (2025) *PagALKBH1A*-mediated m⁶A demethylation reduces the resistance of 84 K poplar to cadmium stress. *Ind. Crop. Prod.* 235, 121831
45. Wang, H. *et al.* (2025) HyperTRIBE mapping of the RNA m⁶A demethylase ALKBH9 binding sites in bamboo reveals its role in plant defense. *Plant Physiol.* 199, kia457
46. Bao, R. *et al.* (2025) Overexpression of the apple m⁶A demethylase gene *MdALKBH1A* regulates resistance to heat stress and fixed-carbon starvation. *J. Integr. Agric.* 24, 1489–1502
47. Li, T. *et al.* (2026) RNA demethylase CsALKBH8 enhances postharvest disease resistance of citrus fruit against *Penicillium digitatum* via m⁶A-mediated transcript regulation. *Food Res. Int.* 240, 119579
48. He, X.-J. *et al.* (2009) An effector of RNA-directed DNA methylation in arabidopsis is an ARGONAUTE 4- and RNA-binding protein. *Cell* 137, 498–508
49. Ramanathan, M. *et al.* (2019) Methods to study RNA-protein interactions. *Nat. Methods* 16, 225–234
50. Ule, J. *et al.* (2003) CLIP identifies Nova-regulated RNA networks in the brain. *Science* 302, 1212–1215
51. McMahon, A.C. *et al.* (2016) TRIBE: hijacking an RNA-editing enzyme to identify cell-specific targets of RNA-binding proteins. *Cell* 165, 742–753
52. Zambelli, F. and Pavesi, G. (2015) RIP-Seq data analysis to determine RNA-protein associations. *Methods Mol. Biol.* 1269, 293–303
53. Gagliardi, M. and Matarazzo, M.R. (2016) RIP: RNA immunoprecipitation. *Methods Mol. Biol.* 1480, 73–86
54. Niranjankumari, S. *et al.* (2002) Reversible cross-linking combined with immunoprecipitation to study RNA-protein interactions in vivo. *Methods* 26, 182–190
55. Zhou, L. *et al.* (2019) RNA methylomes reveal the m⁶A-mediated regulation of DNA demethylase gene *SIDML2* in tomato fruit ripening. *Genome Biol.* 20, 156
56. Li, B. *et al.* (2024) TMK4-mediated FIP37 phosphorylation regulates auxin-triggered N⁶-methyladenosine modification of auxin biosynthetic genes in *Arabidopsis*. *Cell Rep.* 43, 114597
57. Licatalosi, D.D. *et al.* (2008) HTS-CLIP yields genome-wide insights into brain alternative RNA processing. *Nature* 456, 464–469
58. Hafner, M. *et al.* (2010) Transcriptome-wide identification of RNA-binding protein and microRNA target sites by PAR-CLIP. *Cell* 141, 129–141
59. König, J. *et al.* (2010) iCLIP reveals the function of hnRNP particles in splicing at individual nucleotide resolution. *Nat. Struct. Mol. Biol.* 17, 909–915
60. Van Nostrand, E.L. *et al.* (2016) Robust transcriptome-wide discovery of RNA-binding protein binding sites with enhanced CLIP (eCLIP). *Nat. Methods* 13, 508–514
61. Kargapova, Y. *et al.* (2017) sCLIP—an integrated platform to study RNA-protein interactomes in biomedical research: identification of CSTF2tau in alternative processing of small nuclear RNAs. *Nucleic Acids Res.* 45, 6074–6086
62. Zarnegar, B.J. *et al.* (2016) irCLIP platform for efficient characterization of protein-RNA interactions. *Nat. Methods* 13, 489–492
63. Xu, W. *et al.* (2018) Mechanistic implications of enhanced editing by a HyperTRIBE RNA-binding protein. *RNA* 24, 173–182
64. Wang, H. *et al.* (2025) The RNA m⁶A methyltransferase PheMTA1 and PheMTA2 of moso bamboo regulate root development and resistance to salt stress in plant. *Plant Cell Environ.* 48, 5184–5197
65. Amara, U. *et al.* (2022) ALKBH9C, a potential RNA m⁶A demethylase, regulates the response of *Arabidopsis* to abiotic stresses and abscisic acid. *Plant Cell Environ.* 45, 3566–3581
66. Liufu, Y. *et al.* (2023) Inhibition of DNA and RNA methylation disturbs root development of moso bamboo. *Tree Physiol.* 43, 1653–1674
67. Li, T. *et al.* (2023) Comprehensive profiling of epigenetic modifications in fast-growing Moso bamboo shoots. *Plant Physiol.* 191, 1017–1035
68. Wang, Y. *et al.* (2020) Profiling of circular RNA N⁶-methyladenosine in Moso bamboo (*Phyllostachys edulis*) using nanopore-based direct RNA sequencing. *J. Integr. Plant Biol.* 62, 1823–1838
69. Zhang, Y. *et al.* (2023) Transcriptome-wide m⁶A methylation in natural yellow leaf of *Catalpa fargesii*. *Front. Plant Sci.* 14, 1167789
70. Wang, S. *et al.* (2023) m⁶A mRNA modification promotes chilling tolerance and modulates gene translation efficiency in *Arabidopsis*. *Plant Physiol.* 192, 1466–1482
71. Hu, J. *et al.* (2021) N⁶-methyladenosine mRNA methylation is important for salt stress tolerance in *Arabidopsis*. *Plant J.* 106, 1759–1775
72. Cai, J. *et al.* (2024) FIONA1-mediated mRNA m⁶A methylation regulates the response of *Arabidopsis* to salt stress. *Plant Cell Environ.* 47, 900–912
73. Guo, T. *et al.* (2023) The m⁶A reader MhYTP2 regulates the stability of its target mRNAs contributing to low nitrogen tolerance in apple (*Malus domestica*). *Hortic. Res.* 10, uhad094
74. Yue, J. *et al.* (2023) Methyltransferase-like (METTL) homologues participate in *Nicotiana benthamiana* antiviral responses. *Plant Signal. Behav.* 18, 2214760
75. Yue, J. *et al.* (2022) ALKB RNA demethylase homologues and N⁶-methyladenosine are involved in *Potyvirus* infection. *Mol. Plant Pathol.* 23, 1555–1564
76. Zhang, T. *et al.* (2022) N⁶-methyladenosine RNA modification promotes viral genomic RNA stability and infection. *Nat. Commun.* 13, 6576
77. Martinez-Perez, M. *et al.* (2017) *Arabidopsis* m⁶A demethylase activity modulates viral infection of a plant virus and the m⁶A abundance in its genomic RNAs. *Proc. Natl. Acad. Sci. U. S. A.* 114, 10755–10760
78. Bodi, Z. and Fray, R.G. (2017) Detection and quantification of N⁶-methyladenosine in messenger RNA by TLC. *Methods Mol. Biol.* 1562, 79–87
79. Nagarajan, A. *et al.* (2019) Dot blot analysis for measuring global N⁶-methyladenosine modification of RNA. *Methods Mol. Biol.* 1870, 263–271
80. Wang, Y. and Jia, G. (2020) Detection methods of epitranscriptomic mark N⁶-methyladenosine. *Essays Biochem.* 64, 967–979
81. Dominissini, D. *et al.* (2012) Topology of the human and mouse m⁶A RNA methylomes revealed by m⁶A-seq. *Nature* 485, 201–206
82. Meyer, K.D. *et al.* (2012) Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. *Cell* 149, 1635–1646
83. Ke, S. *et al.* (2015) A majority of m⁶A residues are in the last exons, allowing the potential for 3' UTR regulation. *Genes Dev.* 29, 2037–2053
84. Linder, B. *et al.* (2015) Single-nucleotide-resolution mapping of m⁶A and m⁶Am throughout the transcriptome. *Nat. Methods* 12, 767–772
85. Dierks, D. *et al.* (2021) Multiplexed profiling facilitates robust m⁶A quantification at site, gene and sample resolution. *Nat. Methods* 18, 1060–1067
86. Gao, Y. *et al.* (2021) Quantitative profiling of N⁶-methyladenosine at single-base resolution in stem-differentiating xylem of *Populus trichocarpa* using Nanopore direct RNA sequencing. *Genome Biol.* 22, 22
87. Liu, C. *et al.* (2023) Absolute quantification of single-base m⁶A methylation in the mammalian transcriptome using GLORI. *Nat. Biotechnol.* 41, 355–366
88. Hu, L. *et al.* (2022) m⁶A RNA modifications are measured at single-base resolution across the mammalian transcriptome. *Nat. Biotechnol.* 40, 1210–1219
89. Wang, P. *et al.* (2025) Small-molecule-catalysed deamination enables transcriptome-wide profiling of N⁶-methyladenosine in RNA. *Nat. Chem.* 17, 1042–1052
90. Leger, A. *et al.* (2021) RNA modifications detection by comparative Nanopore direct RNA sequencing. *Nat. Commun.* 12, 7198
91. Liu, H. *et al.* (2019) Accurate detection of m⁶A RNA modifications in native RNA sequences. *Nat. Commun.* 10, 4079
92. Sun, H. *et al.* (2025) Mild and ultrafast GLORI enables absolute quantification of m⁶A methylome from low-input samples. *Nat. Methods* 22, 1226–1236

93. Garcia-campos, M.A. *et al.* (2019) Deciphering the “m⁶A code” via antibody-independent quantitative profiling. *Cell* 178, 731–747.e16
94. Zhang, Z. *et al.* (2019) Single-base mapping of m⁶A by an antibody-independent method. *Sci. Adv.* 5, eaax0250
95. Wang, Y. *et al.* (2020) Antibody-free enzyme-assisted chemical approach for detection of N⁶-methyladenosine. *Nat. Chem. Biol.* 16, 896–903
96. Xiao, Y.-L. *et al.* (2023) Transcriptome-wide profiling and quantification of N⁶-methyladenosine by enzyme-assisted adenosine deamination. *Nat. Biotechnol.* 41, 993–1003
97. Bushkin, G.G. *et al.* (2019) m⁶A modification of a 3' UTR site reduces RME1 mRNA levels to promote meiosis. *Nat. Commun.* 10, 3414
98. Anzalone, A.V. *et al.* (2019) Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature* 576, 149–157
99. Lin, Q. *et al.* (2020) Prime genome editing in rice and wheat. *Nat. Biotechnol.* 38, 582–585
100. Wilson, C. *et al.* (2020) Programmable m⁶A modification of cellular RNAs with a Cas13-directed methyltransferase. *Nat. Biotechnol.* 38, 1431–1440
101. Shi, C. *et al.* (2024) Programmable RNA N⁶-methyladenosine editing with CRISPR/dCas13a in plants. *Plant Biotechnol. J.* 22, 1867–1880
102. Yu, L. *et al.* (2024) CRISPR/dCas13(Rx) derived RNA N⁶-methyladenosine (m⁶A) dynamic modification in plant. *Adv. Sci. (Weinh.)* 11, e2401118
103. Wang, N. *et al.* (2017) Functions of two *Malus hupehensis* (Pamp.) Rehd. YTPs (MhYTP1 and MhYTP2) in biotic- and abiotic-stress responses. *Plant Sci.* 261, 18–27
104. Liu, C. *et al.* (2019) Overexpression of *MhYTP2* enhances apple water-use efficiency by activating ABA and ethylene signaling. *Environ. Exp. Bot.* 157, 260–268
105. Jin, Y. *et al.* (2023) Development of an efficient expression system with large cargo capacity for interrogation of gene function in bamboo based on bamboo mosaic virus. *J. Integr. Plant Biol.* 65, 1369–1382
106. Wu, L. *et al.* (2025) Bamboo mosaic virus-mediated transgene-free genome editing in bamboo. *New Phytol.* 245, 1810–1816
107. Wu, L. *et al.* (2026) BaMV-vectored compact AsCas12f1-HKRA enables transgene-free genome editing in Moso bamboo (*Phyllostachys edulis*). *Plant Biotechnol. J.* 24, 2220–2222