Review Article

**m^6A-mediated regulation of crop development and stress responses**

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**Abstract**

Dynamic chemical modifications in eukaryotic messenger RNAs (mRNAs) constitute an essential layer of gene regulation, among which N6-methyladenosine (m^6A) was unveiled to be the most abundant. m^6A functionally modulates important biological processes in various mammals and plants through the regulation of mRNA metabolism, mainly mRNA degradation and translation efficiency. Physiological functions of m^6A methylation are diversified and affected by intricate sequence contexts and m^6A machineries. A number of studies have dissected the functional roles and the underlying mechanisms of m^6A modifications in regulating plant development and stress responses. Recently, it was demonstrated that the human FTO-mediated plant m^6A removal caused dramatic yield increases in rice and potato, indicating that modulation of m^6A methylation could be an efficient strategy for crop improvement. In this review, we summarize the current progress concerning the m^6A-mediated regulation of crop development and stress responses, and provide an outlook on the potential application of m^6A epitranscriptome in the future improvement of crops.

**Introduction**

Posttranscriptional or cotranscriptional RNA chemical modifications play essential roles in determining mRNA fates. Among the 160 distinct mRNA modifications identified so far, N^6^-methyladenosine (m^6A) is the most abundant and best-characterized (Shao et al., 2021). m^6A methylation is reversible in vivo, and functions under the synergistic effect of m^6A methyltransferases (writers), demethylases (erasers) and reader proteins (readers), which install, remove and recognize m^6A marks, respectively (Shi et al., 2019a). In mammals, m^6A installation is mainly implemented by a methyltransferase complex, in which the METTL3/METTL14 (methyltransferase-like 3/14) heterodimer constitutes the core component (Liu et al., 2014; Wang et al., 2016a, 2016b). METTL14 harbours the methyl-transfer activity and functions as a catalytic subunit, while METTL14 facilitates the binding of the complex to the targeted transcripts (Wang et al., 2016a, 2016b). In addition, other subunits including Wilm’s tumour 1-associating protein (WTAP) (Ping et al., 2014), Vir like m^6A methyltransferase associated (VirMA) (Yue et al., 2018), Zinc finger CCCH domain-containing protein 13 (ZC3H13) (Wen et al., 2018) and RNA-binding motif protein 15 (RBM15) (Pati et al., 2016) were also revealed to be functionally important for the m^6A deposition. As for m^6A removal, the fat mass and obesity-associated protein (FTO) and the alkylated DNA repair protein AlkB homolog 5 (ALKBH5), both containing the AlkB domain, represent the well-studied demethylases in mammals (Jia et al., 2011; Zheng et al., 2013). m^6A methyltransferases and demethylases act cooperatively to bring the reversibility for m^6A methylation, accompanied by diversified deposition and distribution along transcripts (Shi et al., 2019a). To exert the physiological functions, m^6A needs to be recognized by the reader proteins. Mammalian YTH-domain proteins, including YTHDF1/2/3 (Dominissini et al., 2012; Wang et al., 2014, 2015) and YTHDC1/2 (Hsu et al., 2017; Xiao et al., 2016), as well as some specific ribonucleoproteins (Alarcón et al., 2015; Liu et al., 2015) or RNA binding proteins (RBPs) (Edupuganti et al., 2017; Huang et al., 2018; Wu et al., 2019), have the ability to recognize m^6A sites, and further modulate the m^6A-modified transcripts by interacting with other functional regulatory proteins, thus were identified as the m^6A reader proteins (Shi et al., 2019a).

Currently, m^6A has been unveiled to functionally modulate mRNA metabolism including mRNA stability (Huang et al., 2018; Wang et al., 2014), translation efficiency (Wang et al., 2015), alternative splicing (Zhao et al., 2014), and nuclear-cytoplasm transport (Roundtree et al., 2017), thereby regulating multiple biological processes, such as embryonic and post-embryonic development (Batista et al., 2014), cell circadian rhythms (Fustin et al., 2013), and cancer stem cell proliferation (Zhang et al., 2016). Given its prevalence and function diversity, m^6A has also been extensively investigated, as an important epigenetic modification, in plants including Arabidopsis and various crops with important agronomic traits (Liang et al., 2020; Shao et al., 2021; Yue et al., 2019).

In the model plant Arabidopsis thaliana, the m^6A machineries have been adequately identified and proved to harbour multiple physiological roles (Shao et al., 2021). The m^6A methyltransferase complex regulates a variety of essential growth and development
processes including embryo development (Zhong et al., 2008), root vascular formation (Růžička et al., 2017), seedling growth (Růžička et al., 2017) and apical dominance formation (Bodi et al., 2012). Importantly, the core subunit of m6A methyltransferase complex, FKBP12 interacting protein 37 KD (RIP37), which is a homolog of the mammalian WTAP, participates in maintaining the normal proliferation of shoot meristems by negatively regulating the mRNA stability of several key shoot meristem genes (Shen et al., 2016). The m6A demethylase AALKBH10B-mediated m6A removal elevates the mRNA stability of flower-promoting genes, thereby positively regulating floral transition (Duan et al., 2017). These studies reveal that Arabidopsis m6A modification has a capacity to decrease mRNA stability, thereby reducing the mRNA abundance of specific genes. However, the YTH-domain protein evolutionarily conserved C-terminal region 2 (ECT2) was shown to stabilize m6A-modified transcripts, and further modulate the development of trichome morphology (Arribas-Hernández et al., 2018; Scutenaire et al., 2018; Wei et al., 2018). Another YTH-domain protein CPSF30-L (the longer isoform of cleavage and polyadenylation specificity factor 30) is involved in regulating the alternative polyadenylation (Hou et al., 2021; Pontier et al., 2019; Song et al., 2021). Therefore, Arabidopsis m6A machineries could adopt diverse molecular mechanisms to cope with divergent physiological processes. Besides the development regulation, m6A modification also mediates the biotic and abiotic stress responses in Arabidopsis, partially by affecting the mRNA stability and alternative polyadenylation of targeted transcripts (Shao et al., 2021). The complexity of m6A functions and mechanisms implies that more pervasive investigations are needed to better understand its biological role in plants. The current mechanistic studies based on Arabidopsis provide an advantageous reference for other plant species including crops.

More recently, the human FTO-mediated plant m6A demethylation caused a ~50% increase in field yield and biomass of rice and potato (Yu et al., 2021). These findings are spectacular and suggest that modulation of m6A methylation may hold potential in serving as a strategy to dramatically improve crop growth and yield. In this review, we summarize the current progress in understanding the m6A characteristics, the m6A-mediated regulation of mRNA metabolism, and the mechanistic links of m6A with developmental processes and stress responses in crops. We also provide an outlook on potential applications and remaining challenges concerning m6A epitranscriptome in the future crop improvement.

m6A characteristics in crops

m6A distribution along transcripts in crops

With the application of high-throughput m6A sequencing technology (m6A-seq) (Dominnisi et al., 2012; Meyer et al., 2012), the transcript-specific m6A localization and enrichment have been uncovered at the transcriptome-wide level in various plant species. In Arabidopsis, thousands of transcripts contain m6A modifications, which distribute preferentially around the start codon or in the 3′ untranslated region (UTR) (Luo et al., 2014; Wan et al., 2015). This distribution preference is conserved among several important crops, including rice (Oryza sativa) (Cheng et al., 2021; Zhang et al., 2019a), maize (Zea mays) (Miao et al., 2020), wheat (Triticum aestivum) (Zhang et al., 2021b), tomato (Solanum lycopersicum) (Hu et al., 2021b; Yang et al., 2021; Zhou et al., 2019), and sweet sorghum (Sorghum bicolor) (Zheng et al., 2021) (Figure 1). A recent study that compared the m6A methylomes for 13 representative plant species spanning over half a billion years of evolution confirmed the conserved distribution of m6A modifications in the stop codon and 3′ UTR regions (Miao et al., 2022). Specially, the m6A modification in strawberry (Fvesca vesca) could also be highly enriched in the coding sequence (CDS) region adjacent to the start codon, besides the occurrence in the stop codon and 3′ UTR region (Zhou et al., 2021) (Figure 1). This unique CDS preference appears in the ripe strawberry fruit, but not the unripe fruit, indicating it is a ripening-specific m6A characteristic (Zhou et al., 2021). Moreover, m6A modifications in apple (Malus domestica) and pak-choi (Brassica rapa) leaves are most abundant in the CDS region, followed by the 3′ UTR region (Guo et al., 2021; Liu et al., 2020) (Figure 1). Therefore, m6A distribution around the stop codon or in the 3′ UTR could be conserved among various plants including Arabidopsis, rice, maize, wheat, tomato, sweet sorghum, strawberry, apple, and pak-choi, while m6A deposition in the CDS region might be development stage-specific or tissue-specific. The inducements related to this distribution characteristic currently remain elusive. A recent study in Arabidopsis suggests that H3K36me2 histone marks contribute to the preferential m6A deposition in the 3′ UTR (Shim et al., 2020). This finding provides us valuable considerations in investigating the relevant mechanisms of m6A distribution in crops.

m6A motifs in crops

The initial m6A-seq analysis revealed that Arabidopsis m6A methylation occurs in a sequence context as RRACH (R represents adenosine (A) or guanosine (G); H represents A, cytidine (C), or uridine (U)) (Duan et al., 2017; Luo et al., 2014), the conserved m6A motif among mammals (Dominissini et al., 2012; Meyer et al., 2012). However, a subsequent study identified a plant-specific m6A motif URRUAH in Arabidopsis, which mainly locates within 3′ UTR and is targeted by the reader protein ECT2 (Wei et al., 2018). These results suggest that Arabidopsis possesses two different m6A motifs. Notably, a recent study claimed that URRUAH is not an m6A motif, but it is rather enriched in the periphery of the canonical RRACH motifs (Arribas-Hernández et al., 2021a). m6A marks in rice (Zhang et al., 2019a), maize (Miao et al., 2020), wheat (Zhang et al., 2021b), or tomato (Hu et al., 2021b; Yang et al., 2021; Zhou et al., 2019) fall into both the RRACH and URRUAH motifs, as the model plant Arabidopsis (Figure 1). Strawberry (Zhou et al., 2021) and sweet sorghum (Zheng et al., 2021) harbour the conserved RRACH motif, while apple (Guo et al., 2021) was demonstrated to possess the plant-specific URRUAH motif (Figure 1). It is possible that the two distinct motifs may extensively exist in most of the crops, and could be individually identified in specific biological processes. Moreover, the consensus sequence of m6A in pak-choi appears to be AAACCV (V means U, A, or G) (Liu et al., 2020), and four new m6A motifs were identified in rice anther, in which the WKUAH (W represents U or A; K means G or U) is the most abundant (Ma et al., 2021) (Figure 1). These findings suggest that m6A modifications in crops involve complicated sequence preferences. However, we currently do not know how m6A machineries achieve selectivity toward certain motif sequences to accomplish m6A installation, removal and recognition. One likely scenario is that they may be localized to diverse sequence contexts through
interaction with RBPs that recognize distinct features of the transcripts.

**Regulation of m^6^A on mRNA metabolism in crops**

m^6^A possesses multiple regulatory effects on mRNA metabolism in Arabidopsis, such as mRNA stability (Anderson et al., 2018; Arribas-Hernández et al., 2021b; Duan et al., 2017; Kramer et al., 2020; Shen et al., 2016; Wei et al., 2018), transcriptome integrity (Pontier et al., 2019), and alternative polyadenylation (Hou et al., 2021; Hu et al., 2021a; Parker et al., 2020; Song et al., 2021). Currently, substantial progresses have been made in deciphering the influence of m^6^A methylation on crop mRNA metabolism, mainly mRNA degradation and translation (Shao et al., 2021).

**Modulation of mRNA stability in crops**

Through the combination of transcriptome-wide m^6^A-seq and RNA-seq analyses, the potential relationship between m^6^A modification and mRNA abundance has been revealed in rice (Cheng et al., 2021), maize (Du et al., 2020; Luo et al., 2020), tomato (Zhou et al., 2019), strawberry (Zhou et al., 2021), and pak-choi (Liu et al., 2020) under divergent physiological circumstances. In the rice root threatened with cadmium stress (Cheng et al., 2021) or pak-choi seedling treated with hot temperature (Liu et al., 2020), no exact correlation between m^6^A modification and mRNA abundance was determined, although thousands of transcripts exhibited differential m^6^A enrichment or gene expression under these stress conditions. However, a positive correlation between m^6^A methylation and mRNA levels was discovered in maize embryos cultured with 2,4-D, an auxin analogue (Du et al., 2020). Moreover, m^6^A modification locating within the stop codon or 3' UTR regions was shown to negatively regulate the mRNA abundance in normally growing maize seedling (Luo et al., 2020), tomato fruit (Zhou et al., 2019), and strawberry fruit (Zhou et al., 2021), while m^6^A enriching in the CDS region in ripe strawberry fruit tends to positively regulate the mRNA levels (Zhou et al., 2021). The different influences of m^6^A modification on mRNA abundance are correlated with the distinct effects of m^6^A on mRNA stability (Guo et al., 2021; Zhou et al., 2019, 2021). m^6^A deposition around the stop codon or within the 3' UTR region possesses the ability to decrease mRNA stability, while m^6^A in the CDS region promotes mRNA stability. However, the underlying mechanisms need to be elucidated.

**Figure 1** m^6^A motifs and distribution preferences along transcripts in various tissues of Arabidopsis and crops. R represents adenosine (A) or guanosine (G); H represents A, cytidine (C), or uridine (U); W represents A or U; K represents G or U; V represents A, G, or U. CDS, coding sequence. UTR, untranslated region. m^6^A motifs were predicted from m^6^A-seq datasets that were performed with at least two independent biological replicates with standard m^6^A-seq procedures.

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Modulation of translation efficiency in crops

Although it is currently unclear whether m6A modification participates in mediating mRNA translation efficiency in Arabidopsis, several studies in crops obtained affirmative answers on this issue. In maize seedling, transcriptome-wide polysome profiling analysis revealed that m6A possesses different effects on translation efficiency, depending on m6A abundances and positions in transcripts (Luo et al., 2020). Concretely, m6A modification nearby the start codon or in the 3' UTR with low strength (low m6A enrichment value) tends to enhance mRNA translation, while m6A with excessive deposition (excessive m6A enrichment value) in the 3' UTR decreases the translation efficiency (Luo et al., 2020). In strawberry fruit and apple leaf, m6A methylation was also demonstrated to facilitate mRNA translation (Guo et al., 2021; Zhou et al., 2021). More recently, the rice m6A adenosine methylase 2 (OsMTA2) was revealed to interact with the eukaryotic translation initiation factor 3 subunit h (OsEIF3h) (Huang et al., 2021), implying that m6A modification may modulate mRNA translation via the interactions between m6A machineries and translation factors, adopting the analogous molecular mechanism as the mammals (Lin et al., 2016; Wang et al., 2015).

Regulatory mechanisms of m6A on crop developmental processes

Arabidopsis m6A marks have been demonstrated to modulate multiple physiological processes, reflecting its functional diversity in controlling plant development (Liang et al., 2020; Shao et al., 2021). Disruption of m6A machineries including m6A methyltransferase, demethylases and reader proteins could be an effective pointcut to explore the functional significance of m6A in both Arabidopsis and crops. Current researches have uncovered the m6A-mediated regulation of key genes with critical roles in crop growth and development.

Regulation of development in grain crops

OsFIP, a homolog of the mammalian WTAP, was identified as one of the components in the m6A methyltransferase complex in rice (Zhang et al., 2019a). Functional investigation revealed that OsFIP mediates m6A deposition on transcripts of sporogenesis-related genes encoding the NTPases and threonine proteases, leading to the acceleration of degradation of these transcripts to control microsporogenesis (Zhang et al., 2019a) (Figure 2a). Deficiency of the rice m6A methyltransferase-like domain-containing protein enhances downy mildew 2-like (OsEDM2L) impaired tapetal programmed cell death (PCD) and causes defective pollen development, indicating its indispensable role for normal anther development (Ma et al., 2021). OsEDM2L could not only interact with the transcription factors basic helix-loop-helix 142 (bHLH142) and tapetum degeneration retardation (TDR) to activate the expression of eternal tapetum 1 (EAT1), a positive regulator of tapetal PCD and anther development (Ma et al., 2021) (Figure 2b). Moreover, the interaction of OsMTA2 with the OsEIF3h suggests that OsMTA2 may participate in regulating OsEIF3h-mediated seedling growth and pollen development in rice, although the molecular mechanism is poorly understood (Huang et al., 2021).

In maize, m6A-mediated post-transcriptional regulation contributes to the heterosis in hybrid seedlings and the induction of callus cultured with the addition of auxin analogue 2,4-D (Du et al., 2020; Luo et al., 2021). The former was predicted to correlate with the increased translation efficiency of m6A-modified transcripts (Luo et al., 2021), while the latter may be caused by the elevated mRNA abundance of several key genes involved in the callus induction (Du et al., 2020).

Regulation of development in horticultural crops

Fruit expansion represents an important process for fruit growth and development. It has been revealed that the overall m6A level increases during tomato fruit expansion, accompanied by the elevated m6A enrichments and transcript levels in several essential expansion-related genes (Hu et al., 2021b). Exogenous treatment by 3-deazaneplanocin A (m6A writer inhibitor) or meclofenamic acid (m6A eraser inhibitor) suppresses the expansion of fruit (Hu et al., 2021b), suggesting that m6A methylation participates in modulating the growth and development of tomato fruit. However, the underlying molecular mechanisms need to be clarified.

Recently, m6A methylation was reported to regulate fruit ripening, an important biological process for quality formation (Zhou et al., 2019, 2021). In the climacteric fruit tomato, m6A demethylase SIALKBH2 targets the DNA demethylase gene DEMETER-like DNA demethylase 2 (SIDML2), a key ripening-promoting gene, and positively modulates its expression by elevating mRNA stability, thus accelerating tomato fruit ripening. Interestingly, SIDML2 can in turn act on SIALKBH2 to activate its transcription by the repressive effect on DNA methylation (5-methylcytosine, 5mC). These results suggest that SIALKBH2 and SIDML2 function synergistically during tomato fruit ripening, representing an internal correlation between m6A and 5mC (Zhou et al., 2019) (Figure 2c). In the non-climacteric fruit strawberry, the m6A methyltransferase FvMTA was demonstrated to positively regulate fruit ripening (Zhou et al., 2021). FvMTA-mediated m6A modification increases mRNA stability or translation efficiency of key genes in the ABA pathway including 9-cis-epoxycarotenoid dioxygenase 5 (FvNCED5), ABA-responsive element-binding protein 1 (FvAREB1) and putative ABA receptor (FvABAR), which are essential for the ripening of strawberry fruit (Figure 2d). Strawberry genome contains four SIDML2 homologs, among which two contain differential m6A peaks at the onset of fruit ripening. However, none of these genes exhibits a significant increase in mRNA abundance upon ripening initiation. In addition, no differential m6A modification was observed in the transcripts of DNA methyltransferase genes in the RNA-directed DNA methylation (RdDM) pathway, which governs the reprogramming of DNA methylation during the ripening of strawberry fruit. This suggests that DNA methylation is dispensable for the FvMTA-mediated fruit ripening in strawberry (Zhou et al., 2021). Thus, regulation of fruit ripening via m6A modification involves complicated molecular mechanisms and could be distinct among various fruits.

Regulatory mechanisms of m6A on crop stress responses

Mutation of m6A writers in Arabidopsis induces significant changes in the expression of genes responsive to abiotic and biotic stresses, as revealed by transcriptome analysis (Anderson et al., 2018; Bodi et al., 2012; Hu et al., 2021a). Accordingly, the m6A-mediated stress responses have been extensively studied in
various crops, including tobacco (*Nicotiana tabacum*) (Li et al., 2018), rice (Cheng et al., 2021; Shi et al., 2019b; Tian et al., 2021; Zhang et al., 2021a), wheat (Sun et al., 2020; Zhang et al., 2021b), sweet sorghum (Zheng et al., 2021), tomato (Yang et al., 2021), apple (Guo et al., 2021; Mao et al., 2021), and pakchoi (Liu et al., 2020).

Figure 2 Function of m^6^A modification on crop growth and development or stress resistance. (a) Regulation of rice m^6^A methyltransferase subunit OsFIP on microspore development. OsFIP-mediated m^6^A installation decreases the expression of genes encoding the threonine proteases and NTPases, thereby maintaining the normal development of rice microspores. Os, *Oryza sativa*. (b) Regulation of rice m^6^A methyltransferase-like domain-containing protein OsEDM2L on anther development. OsEDM2L-mediated m^6^A installation facilitates the proper alternative splicing and polyadenylation of the OsEAT1 mRNA that encodes a positive regulator of tapetal programmed cell death during the anther development. Moreover, OsEDM2L could directly activate the OsEAT1 transcription by interacting with the transcription factors bHLH142 and TDR. (c) Regulation of tomato m^6^A demethylase SIALKBH2 on fruit ripening. SIALKBH2-mediated m^6^A removal promotes mRNA stability of DNA demethylase gene SDML2, a key ripening-promoting gene, thereby facilitating fruit ripening. SDML2 in turn acts on SIALKBH2 to activate its transcription by DNA demethylation, representing an interplay between m^6^A RNA methylation and DNA methylation during tomato fruit ripening. Sl, *Solanum lycopersicum*. (d) Regulation of strawberry m^6^A methyltransferase FvMTA on fruit ripening. FvMTA-mediated m^6^A installation promotes mRNA stability or translation efficiency of key genes in ABA pathway including FvNCED5, FvAREB1 and FvABAR, thereby facilitating fruit ripening. Fv, *Fvesca vesca*. (e) Regulation of apple m^6^A reader MhYTP2 on leaf resistance to powdery mildew. MhYTP2-mediated m^6^A recognition elevates the mRNA stability of MdBML19, a positive regulator in powdery mildew resistance, and the translation efficiency of antioxidant genes, thereby enhancing the resistance of apple leaves to powdery mildew. Mh, *Malus hupehensis*; Md, *Malus domestica*. (f) Regulation of human m^6^A demethylase FTO on rice root growth and tiller formation. Heterologous expression of human FTO in rice promotes root growth and tiller formation by regulating the expression of genes in various metabolic pathways, therefore facilitating rice yield and biomass.
Regulation of the biotic stresses in crops

Tobacco mosaic virus (TMV) infection causes the increased expression of the potential demethylase genes, concomitant with the decreased expression of the potential methyltransferases, implicating the involvement of m6A in modulating the virus-induced stress responses in tobacco (Li et al., 2018). In rice, the infection of rice stripe virus or rice black-stripe dwarf virus (RBSDV) causes a dramatical increase in overall m6A methylation level, accompanied by the changed transcription in genes encoding m6A machinerys, implying that m6A modification might be involved in the defence response against virus infection (Zhang et al., 2021). Furthermore, significant changes in m6A methylome profile, as well as its correlation with mRNA abundance, have been deciphered in pak-choi seedling under heat stress (Liu et al., 2020), tomato anther under cold stress (Yang et al., 2021), and apple leaf under drought stress (Mao et al., 2021). These results revealed that m6A modification also participates in controlling the responses of crops to temperature and humidity-induced stresses.

Concluding remarks and future outlook

Rapid advances have been made in recent years in understanding the functional diversity of m6A marks, especially in some important biological processes. Physiological effects of m6A on plant development or stress resistance throughout the life cycle facilitate plant adaptation to the complicated and volatile ecological environment. In spite of this, one extremely essential issue is how to employ m6A investigations to increase the yield and quality of crops, known as crop improvement, thereby facilitating human health. Heterologous expression of human m6A demethylase FTO in rice and potato dramatically elevates the yield and biomass through the transcriptional modulation of genes involved in various metabolic pathways (Yu et al., 2021) (Figure 2f). Recent studies revealed that m6A methyltransferase or demethylase participates in modulating tomato and strawberry fruit ripening, an important process for fruit quality formation (Zhou et al., 2019, 2021). Moreover, m6A reader proteins were demonstrated to mediate the stress responses in both wheat and apple (Guo et al., 2021; Sun et al., 2020; Zhang et al., 2021b). These findings highlight the importance of m6A machinerys in physiological regulations, and provide us the possibility for improving crop yield, quality, and stress resistance by controlling the bioactivity of m6A machinerys.

Although m6A modification possesses great potential for crop improvement, there exist several major challenges. Firstly, modulation of the key components in m6A system may cause changes in the overall levels of m6A methylation, leading to unpredictable effects. Therefore, a transcriptome-wide m6A modification map at single-base resolution is necessary for precise m6A editing at specific sites without changing the overall levels of m6A or the primary sequence of genes with critical roles in crop development or stress response (Zheng et al., 2020). However, the current m6A modification maps constructed for plants can only display m6A modification in a range of 100–200 nucleotides. Current advances in high-throughput m6A detection techniques at single-base resolution, including m6AIP (m6A individual-nucleotide-resolution crosslinking and immunoprecipitation) (Linder et al., 2015), MAZTER-seq (RNA digestion via m6A sensitive RNase and sequencing) (Garcia-Campos et al., 2019), m6A-REF-seq (m6A-sensitive RNA-endoribonuclease-facilitated sequencing) (Zhang et al., 2019b), and nanopore DRS (direct RNA sequencing) (Parker et al., 2020; Pratanwanich et al., 2021), may facilitate the precise identification of m6A sites. The second challenge is how to add or remove specific m6A modification sites in gene transcripts. The normal genome editing technique can only result in the addition or deletion of the gene sequences, but not the m6A modification sites. This could be resolved by the application of m6A editing, which makes it possible to precisely reconstruct the m6A marks at specific sites. As a new tool, m6A editing appears to be a fusion of m6A enzymes (writers or erasers) and clustered regularly interspaced short palindromic repeat (CRISPR/Crispr-associated nucleas 9 (Cas9) technology. The m6A editing can add or remove m6A at specific sites under the guidance of single-
guide RNA and protospacer adjacent motif (Cox et al., 2017; Xu et al., 2019; Zheng et al., 2020). Recently, it is proposed that Cas13 has higher RNA target specificity and efficiency than Cas9, and is more suitable for the m^6A editing system (Zheng et al., 2020). Moreover, CRISPR-based prime editing harbours the ability to achieve the substitution, deletion or insertion of all single nucleotides in plant genomes, thus holding great potential in future m^6A editing (Jin et al., 2021). The third challenge is that a number of m^6A machineries, including writers, erasers, and readers, remain uncertain in crops. Although some m^6A enzymes have been identified in several crops, such as rice, apple, and tomato, their numbers are few relative to those identified in animals. Besides the main challenges, many important questions require further explorations for better understanding and application of m^6A in crop improvement. For example, what are the molecular mechanisms underlying the crosstalk among m^6A machineries in the regulation of crop development and stress responses? Whether m^6A participates in regulating the trade-off between growth and resistance? In summary, the m^6A-mediated regulation in crops is an essential and complicated research area with nondeterminacy and many challenges.

In recent years, a series of bioinformatics tools were developed to simplify the analysis of m^6A epitranscriptomics. RNAmodR is the first publicly analytical toolkit suitable for m^6A analysis (Evers et al., 2016). However, this tool focuses on the annotation of m^6A distribution along transcripts, but lacks functions for m^6A site calling. Subsequently, several prediction tools based on mammalian or yeast sequences were developed to predict m^6A sites (Zhai et al., 2018). On that basis, a plant-specific epitranscriptome package, named Plants Epitranscriptome Analysis (PEA), was exploited (Zhai et al., 2018). This bioinformatic toolkit is versatile and could perform the calling, prediction, and functional annotation of m^6A sites produced from both common m^6A-seq and high-throughput m^6A detection techniques at single-base resolution, such as miCLIP (Zhai et al., 2018). RNAmod is another versatile m^6A toolkit, which facilitates the visualization and functional annotation of m^6A modifications in multiple species including Arabidopsis (Liu and Gregory, 2019). However, whether PEA and RNAmod could be applied in numerous crop species needs to be determined. In addition, with the rapid development of m^6A detection techniques, more convenient and integrated bioinformatic toolkits are required to be developed to achieve accurate identification of m^6A sites, for example, those suitable for the third-generation nanopore sequencing are currently vacant in plants.

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Conflict of interest
The authors declare that they have no competing interests.

Authors’ contributions
GQ conceived the topic. LZ, GQ, GG, RT, WW, and YW wrote the manuscript. All authors read and approved the final manuscript.

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