Developmental and Physiological Regulation by Epitranscriptomic Modifications

Subjects: Agriculture, Dairy & Animal Science

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Epitranscriptomic modifications play important roles during plant development and in various responses to biotic and abiotic stresses. The major developmental processes affected by these modifications include organogenesis, embryonic and cotyledon development, seed development and seed yield, root and shoot growth, leaf morphology, trichome branching, floral transition, the proliferation of shoot apical meristem, and fruit ripening.

epitranscriptomics plant development plants methylation genome editing

1. Developmental and Physiological Regulation by Epitranscriptomic Modifications in Plants

Epitranscriptomic modifications play important roles during plant development and in various responses to biotic and abiotic stresses. The major developmental processes affected by these modifications include organogenesis, embryonic and cotyledon development, seed development and seed yield, root and shoot growth, leaf morphology, trichome branching, floral transition, the proliferation of shoot apical meristem, and fruit ripening, as illustrated in **Table 1**.

Table 1. Role of post-transcriptional modifications in plants' growth and development.

Gene	Modification	Developmental Role	Plant Species	Reference
TRM61/TRM6	tRNA (m^1A)	Embryogenesis	*Arabidopsis thaliana*	[1][2]
Complex				
PhTRMT61A	mRNA (m^1A)	leaf development	*Petunia*	[3]
AtTRM5	tRNA	leaf and root development	*Arabidopsis thaliana*	[4]
	(m^1G, m^1I)	flowering time regulastion		
TCTP1	mRNA (m^5C)	root growth	*Arabidopsis thaliana*	[5]
FIP37	mRNA (m^6A)	embryo development	*Arabidopsis thaliana*	[6]
		trichome endoreduplication and shoot stem cell fate		[7][8]

Gene	Modification	Developmental Role	Plant Species	Reference
OsEMD2L	mRNA (m^6A)	anther development	*Oryza sativa*	[9]
ECT2	mRNA (m^6A)	trichome branching, trichome morphology	*Arabidosis thaliana*	[10][11]
MTA, *MTB*, *FIP37*, *VIR*, *HAKAI*	mRNA (m^6A)	vascular formation in roots, pattern formation	*Arabidopsis thaliana*	[12]
MTA, *MTB*	mRNA (m^6A)	fruit ripening	*Fragaria ananassa*	[13]

2. Seed Development

Seed development is a complex process integrating different genetic, metabolic, and physiological pathways regulated by transcriptional, epigenetic, peptide hormone, and sugar regulators [14][15]. The chemical modifications associated with seed development, such as oxidation and methylation in mRNA and genomic DNA, affect gene expression during the later stages of seed development. DNA methylation in Arabidopsis is a dynamic process, and during seed development, there is a drastic increase in the global level of non-CG methylation throughout the seed, whereas CG and CHG-methylations do not change significantly. DNA methylation regulates the maternal expression of *DOG4* and *ALN*, which are the negative regulators of seed dormancy. However, the special methylation marks associated with seed dormancy and the germination transcriptomes remain to be elucidated [16]. MTA, an m^6A mRNA methyltransferase, is essential for embryogenesis, and its homozygous insertional knockout mutant “mta” showed an embryo arrest at the globular stage due to a lack of m^6A at the poly(A) RNA, whereas the hemizygotes produced green and white seeds in immature siliques. However, the complementation lines rescued the embryo-lethal phenotype, indicating that the insertion mutation in *MTA* was embryo-lethal [6][17]. *AtTRM61* and *AtTRM6* cause N1 methylation of adensoine58 (A58) in tRNA, and the loss of function of either of these tRNA methyltransferases causes seed abortion. Mutations in the complex *AtTRM61*/*AtTRM6* subunits result in developmental defects in the embryo and endosperm. However, conditional complementation of At*TRM61* showed that tRNA m1A58 modification is crucial for endosperm and embryo development [18]. *CMAL* is responsible for the methylation of N4-methylcytidine rRNA in the chloroplast and plays a key role in the chloroplast’s function, development, and abscisic acid (ABA) response in Arabidopsis. The loss-of-function *cmal* mutant exhibited a reduction in silique size, the number of seeds per silique, and total seed yield compared with wild-type (WT) plants, indicating its important role in seed development [19].

3. Root Development and Growth

Root development is a critical aspect of plant growth and allows the effective use of water resources. Plants, being sessile by nature, must adapt to various environmental cues. Epitranscriptomic modifications play a crucial role in root development processes. In Arabidopsis, *AtTRM4B* is involved in the methylation of m^5C sites in the root transcriptome and positively regulates its growth through cell proliferation of root apical meristem. A T-DNA

insertion mutant, *trm4b*, had a shorter primary root than the WT. The *trm4b*/*trdmt1* double mutant also exhibited a shorter root phenotype. Furthermore, the *TRM4B* mutant was more sensitive to oxidation stress, implying that *TRM4B* contributes to root growth by regulating the response to oxidative stress [20]. Another study has shown that *TRM4B* contributes to primary and lateral root development in Arabidopsis by regulating the transcript levels of *SHY2* and *IAA16*. The m^5C levels in *TRM4B* were reduced by 20–30% in roots and exhibited a shorter root phenotype; however, its level remains unchanged in aerial tissues [21]. At*TRM5* is a bifunctional guanine and inosine-N1-methyltransferase tRNA and *trm5-1* mutant with reduced levels of m^1G and m^1I and a reduced number of lateral roots and total root length compared with WT plants. However, *TRM5* complementation lines reversed the knockout mutant phenotypes, indicating that *TRM5* is involved in regulating the root development of Arabidopsis [4]. The m^6A writer and reader proteins are highly expressed in the root meristems, apexes, and lateral root primordia [6][12][22]. In poplar, root development is affected by *PtrMTA* and *OE-PtrMTA-14*, *OE-PtrMTA-10*, and *OE-PtrMTA*-6 lines with almost double the m^6A level, exhibiting better root and root tip growth compared with those of WT [23]. Recent research showed that the m^6A level changes in response to ammonium ($NH4^+$) nutrition and regulates the proteome response through altered translation in maritime pine roots [24]. Rice cultivar (cv.9311) exposed to cadmium stress exhibited abnormal root development caused by altered methylation profiles in transcripts involved in various biosynthetic, metabolic, and signaling processes, indicating that m^6A plays an important role in regulating the gene expression level of various cellular pathways [25]. In Arabidopsis, correct m^6A methylation plays an important role in developmental decisions, and *Virilizer-1* (m^6A methyltransferase) plays an important role in maintaining m^6A levels. The deletion of *vir-1* showed aberrant root cap formation and defective protoxylem development, indicating that m^6A is essential for root development [12]. Another study has shown that multi-walled carbon nanotubes inhibit root growth by reducing m^6A levels [26]. In rice, FTO expression increases root apical meristem cell proliferation and modulation of m^6A RNA levels, which is a promising strategy to improve growth. *FTO*-transgenic plants showed a 35% and 45% increase in the total number and length of their lateral roots, respectively, and the number and length of their primary roots increased more than 3.3 fold at the tillering stage compared with the WT plants [27]. The m^6A reader proteins named *ECT2*, *ECT3,* and *ECT4* are highly expressed at the root apex and throughout the lateral root formation. Loss of ECT2 function caused a right-ward tilt in root growth, and the ect2/ect3 double mutants show slower root growth, whereas the ect2/ect3/ect4 triple mutants show agravitropic behavior along with a slower root growth compared with the WT [22]. Genes affecting various plant developmental processes such as floral transition [28][29], seed development [13][16][30], root growth [16][31], leaf growth [22], and fruit ripening [17][32] are illustrated in **Figure 1**.

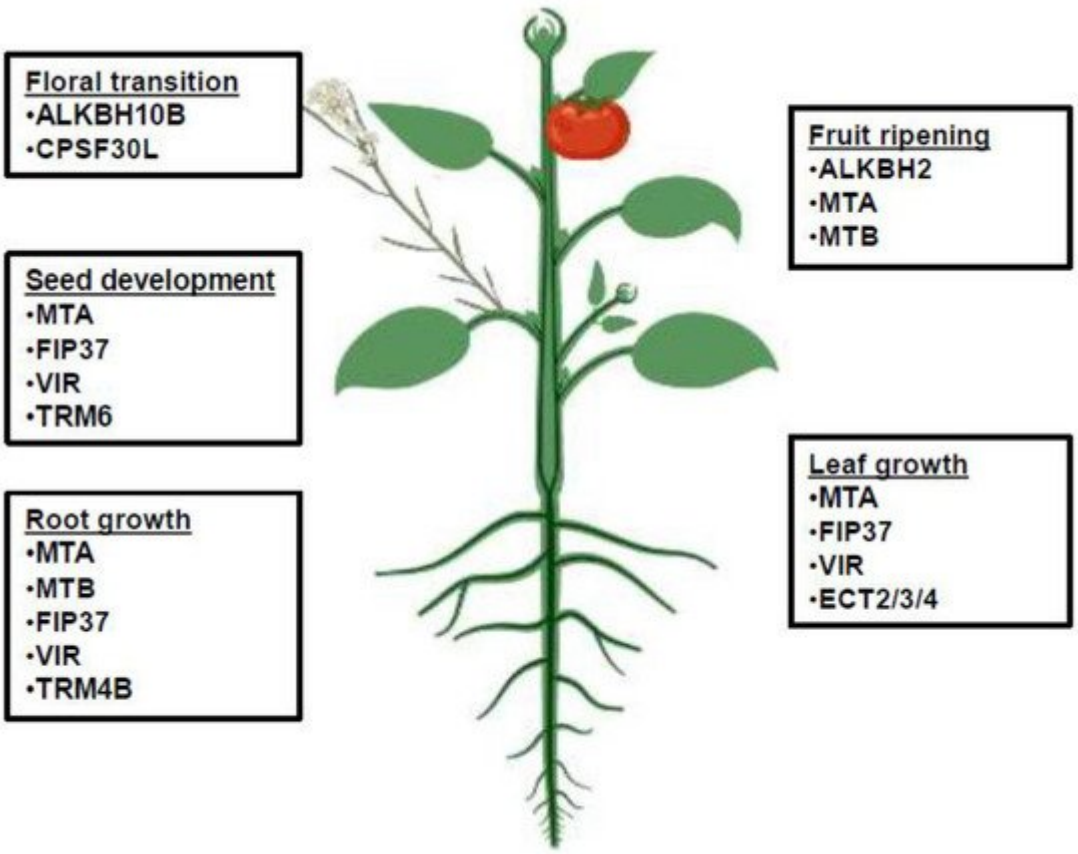

Figure 1. Regulation of plant's development by post-transcriptional modifications (m^6A and m^5C); post-transcriptional modifications regulated by different writer, eraser, and reader proteins affect various plant developmental processes including seed development, leaf and root growth, floral transitions, and fruit ripening.

4. Anther/Pollen Development

Anthers produce male gametes and certain sporophytic and gametophytic tissues in flowering plants. The tapetum of anthers acts as a bridge for nutrient exchange and communication between sporophytic and gametophytic cells. A recent study has shown the involvement of m^6A in anther development in rice. *OsEMD2L* contains an N6-adenine methyltransferase-like (MLT) domain, and the *osemd2l* mutant showed an altered m^6A landscape with Eternal Tapetum 1 (*EAT1*) transcription. The dysregulated alternative splicing and polyadenylation of *EAT1* resulted in the suppression of *OsAP25* and *OsAP37* and led to delayed tapetal-programmed cell death and male sterility [9]. Another study showed that the transgenic expression of *FTO* in rice increased the total number of productive tillers per plant by 42% and improved productivity [27]. *OsFIP* and *OsMTA2* are the components of the m^6A RNA methyltransferase complex in rice. *OsFIP* is essential for male rice gametogenesis and modifies m^6A during sporogenesis by recognizing a panicle-specific "UGWAMH" motif. The *osfip* knockout mutant showed an early degeneration of microspores and abnormal meiosis in prophase I, and had 1.4 tillers per plant compared with 4.7 in WT plants. Furthermore, at the late reproductive stage, *fip* plants were almost sterile and had shorter panicles and reduced seed numbers, and 84.8% of the pollen grains lacked starch, indicating that *OsFIP* plays an important role in microspore development [33]. In tomatoes, the widely spread m^6A modification in anthers is disrupted under cold

stress conditions and affects the expression level of genes involved in tapetum and microspore development. The moderately low-temperature-induced pollen abortion is due to impaired micro gametogenesis, tapetum degeneration, and pollen wall formation. Additionally, m^6A is associated with ABA transport in anthers or sterol accumulation for pollen wall formation, and targets the ATP-binding cassette G gene, *SLABCG31* [34].

5. Floral Regulation

Precise initiation of flowering is essential for plant reproductive success, and several epigenetic modifications play important roles during floral transition. A recent report showed that m^6A-mediated RNA modification was involved in the complex genetic regulation that controls floral regulation. The loss of function of the RNA demethylase, *ALKBH10B,* increased m^6A modification and delayed floral transition due to the increased mRNA decay of the flowering regulator *FT* and its up-regulators, *SPL3,* and *SPL9* [28]. *AtTRM5* encodes nuclear-localized bifunctional tRNA guanine and inosine-*N1*-methyltransferase and is important for growth and development. The loss-of-function *Attrm5* mutant showed an overall slow growth and delayed flowering. At the inflorescence emergence stage, *trm5-1* plants exhibited a reduced number of rosette leaves, smaller leaves, reduced fresh weight, and took longer to flower; however, *TRM5*-overexpressing plants flowered slightly earlier than WT. The delayed flowering phenotype in *trm5-1* mutants was due to a deficiency in floral time regulators, including *GI*, *CO*, and *FT*, the downstream floral meristem identity gene *LEAFY* (*LFY*), and circadian clock-related genes [4]. *CMAL* is a chloroplast-localized rRNA methyltransferase and is responsible for the modification of N4-methylcytidine (m^4C) in 16S chloroplast rRNA. The loss-of-function *cmal* mutant showed stunted growth and delayed flowering due to altered expression levels of various flowering-related genes, including *APETALA1 (AP1)*, *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1)*, *FRUITFULL (FUL)*, *CAULIFLOWER (CAL)*, and *Flowering Locus C (FLC)*; however, the *CMAL* complementation lines recovered stunted growth phenotypes, indicating that stunted growth is due to the lack of m^4C modification [35].

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