Plant Communications, Volume 4

Supplemental information

The plant cytosolic m⁶A RNA methylome stabilizes photosynthesis in

the cold

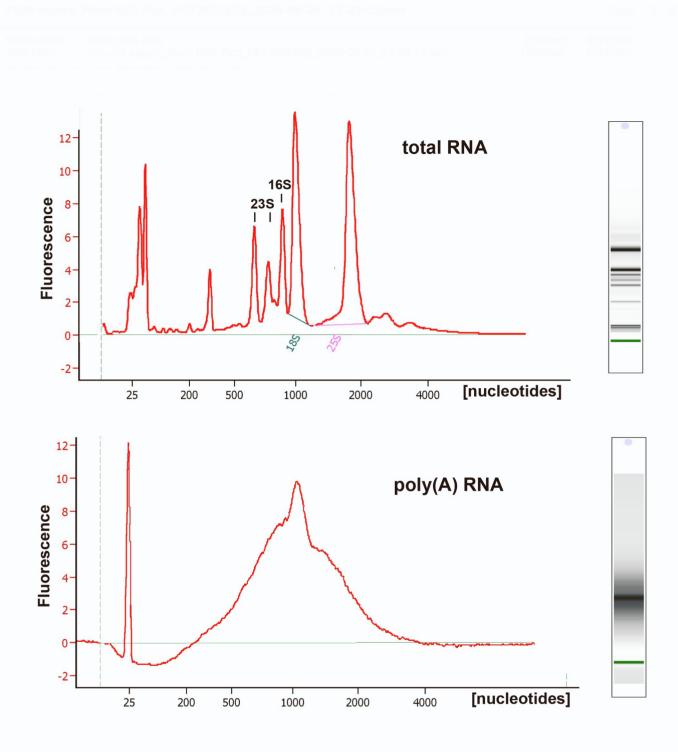
Alexandre Magno Vicente, Nikolay Manavski, Paul Torben Rohn, Lisa-Marie Schmid, Antoni Garcia-Molina, Dario Leister, Charlotte Seydel, Leo Bellin, Torsten Möhlmann, Gregor Ammann, Stefanie Kaiser, and Jörg Meurer **Plant Communications**

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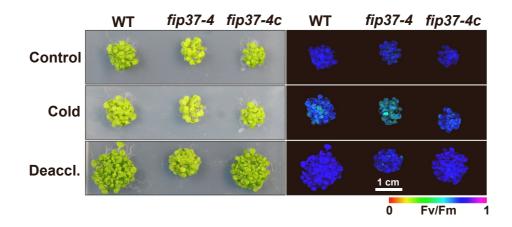
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Supplemental Figures 1-7 Supplemental Tables 1-2



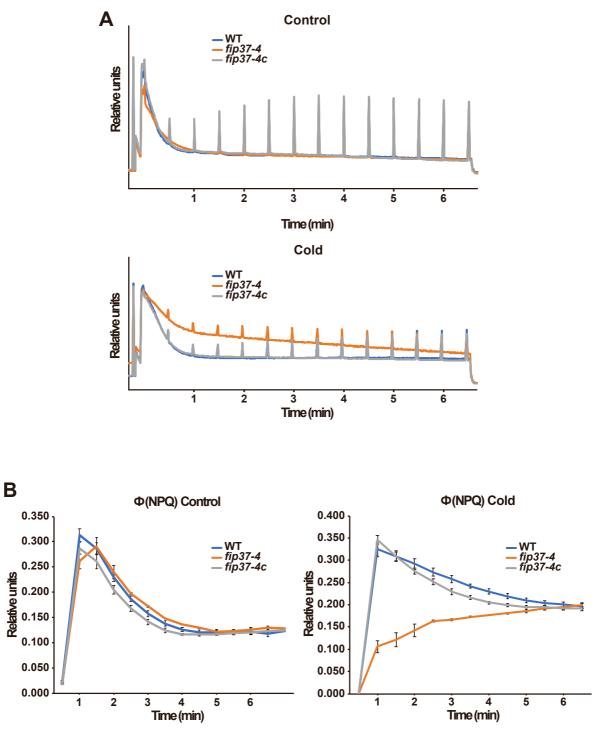
Supplemental Figure S1. Quality control of enriched poly(A)-RNA

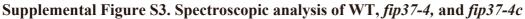
Bioanalyzer electropherograms (RNA Pico 6.000) show representative samples of total (upper part) and poly(A)-enriched (lower part) RNAs used for m⁶A quantification. Cellular rRNAs in total RNAs and their depletion in poly(A)-enriched RNA samples are shown. The presence of cytoplasmic 25S and 18S, and plastid 16S and 23S rRNA peaks indicated good integrity of RNAs. The corresponding virtual gels are shown on the right.



Supplemental Figure S2. FIP37 deficiency strongly but reversibly affects photosynthesis during cold treatment

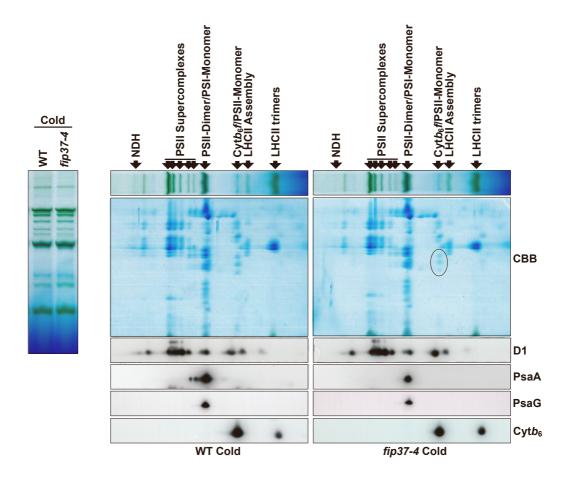
The left part shows the visible phenotype of WT, fip37-4, and fip37-4c grown in vitro for 10 days under standard condition followed by 4 days in the cold and another 4 days of deacclimation. Corresponding chlorophyll fluorescence images reflecting Fv/Fm values are shown on the right. Scale bar: 1 cm.





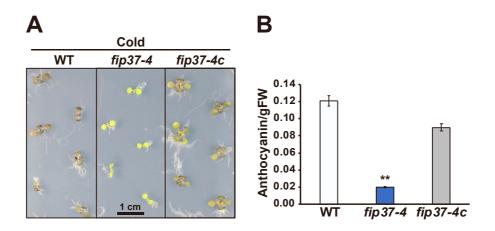
(A) Representative chlorophyll fluorescence induction analysis of WT, *fip37-4*, and *fip37-4c* lines grown for 10 days under control and additional 4 days under cold conditions on soil. The actinic light intensity was set to 80 μ mol photons m⁻² s⁻¹ and consecutive saturation pulses were applied every 30 s.

(B) The corresponding course of $\Phi(NPQ)$ during induction of plants grown under standard and cold conditions is shown.



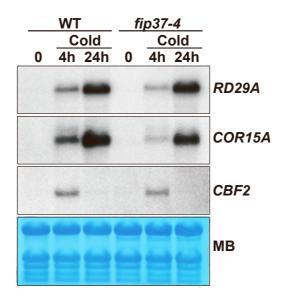
Supplemental Figure S4. Analysis of thylakoid membrane complexes using BN-PAGE

Loading of thylakoid complexes was based on equal amount of chlorophyll. The first dimension was performed using BN-PAGE of solubilized thylakoid membrane complexes of WT and mutant plants grown under cold conditions (left part). The appearing protein complexes are indicated (right upper part). Proteins in the second dimension were stained with Coomassie Brilliant Blue (CBB) (right middle part). The Cyt*b*₆*f* complex is circled. The second dimension was subjected to immunodecoration using D1, PsaA, PsaG, and Cyt*b*₆ sera (right lower part).



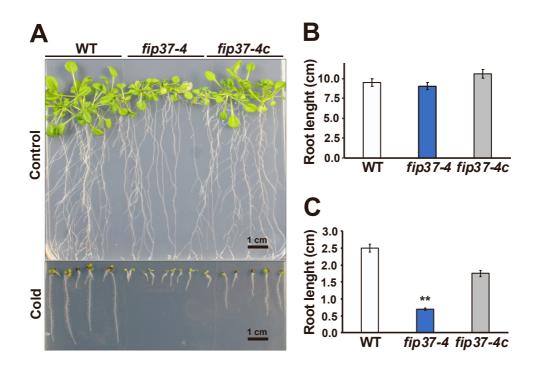
Supplemental Figure S5. FIP37 is important for anthocyanin production in the cold (A) Phenotype of WT, *fip37-4*, and *fip37-4c* grown in vitro at 22°C for 7 days followed by a shift to 4°C for 30 days.

(B) Anthocyanin content in WT, *fip37-4*, and *fip37-4c* after cold treatment. 15 replicates for each genotype were measured. Statistical significance was determined using Tukey HSD. ** corresponds to a p-value ≤ 0.01 . Fresh weight: FW. Scale bar: 1 cm.



Supplemental Figure S6. Reduced levels of FIP37 has no significant impact on the expression of cold-responsive genes

RNA gel blot analysis of *CBF2*, *COR5A*, and *RD29A* genes in the WT and *fip37-4* before (time 0) and after 4 and 24 hours of cold treatment. 8 μ g of RNAs from leaves were used. Methylene blue (MB) was used as loading control.



Supplemental Figure S7. Cold-sensitive root growth in *fip37-4*

(A) Phenotype of WT, *fip37-4*, and *fip37-4c* grown vertically on MS medium for 21 days under control conditions (upper part) and after germination for 30 days at 4°C (lower part). Scale bar: 1 cm.

(**B** and C) Graphs show root lengths of plants grown under control (**B**) and cold (C) conditions. 25 replicates for each genotype were measured. Statistical significance was determined using Tukey HSD. ** corresponds to a p-value ≤ 0.01 .

Supplemental Table S1. List of putative epitranscriptomic players with chloroplast localization or functional association

The functions of potential epitranscriptomic factors were retrieved from TAIR, INTERPRO, or the STRING database (arabidopsis.org, ebi.ac.uk, string-db.org, respectively). The putative subcellular localization was estimated by ARAMEMNON (aramemnon.uni-koeln.de). The consensus score AramLocCon is indicated. The tissue with highest expression levels was taken from the Arabidopsis eFP Browser (<u>http://bar.utoronto.ca</u>). The DOI numbers of latest references (TAIR) dealing either specifically or generally with the factors is listed. Interaction partners were predicted using the STRING and the Biogrid (thebiogrid.org) database.

Gene name	Tentative function	Subcellular localisation and consensus score	Mainly expressed in:	References/DOI	Interactions
black, writers red, readers blue, erasers	Interpro (www.ebi.ac.uk) String (string- db.org) TAIR (arabidopsis.org)	AramLocCon (aramemnon.uni- koeln.de) c, chloroplast m, mitochondrium n, nucleus	Arabidopsis eFP Browser (http://bar. utoronto.ca)	TAIR (arabidopsis.org)	String (string-db.org) Biogrid (thebiogrid.org)
AT1G01860	PFC1, chloroplast Ribosomal RNA adenine dimethylase family protein	c 21.1	Leaves	10.1105/tpc.10.5. 699	AT4G26600, methyltransferase activity, RNA binding; Involved in rRNA processing; AT3G13230, RNA-binding KH domain-containing protein; AT3G11964, rRNA biogenesis protein <i>rrp5</i>
AT1G06560	putative NSUN 5- methylcytosine RNA methyltransferase (AtNOP2c); rRNA processing	c 1.9, n 3.9	Leaves	10.1186/s12870- 017-1206-0	AT5G04600, RNA-binding (RRM/RBD/RNP motifs) family protein
AT1G12800	chloroplast localized S1 RNA binding protein, OB-fold-like protein	c 21.8	Leaves	10.1111/tpj.12889	At1G63310, Uncharacterized protein; AT1G06720, GTPase activity; Involved in ribosome biogenesis; AT3G06530, Involved in ribosome biosynthesis ; AT4G04940, Transducin family protein / WD-40 repeat family protein; Its function is described as nucleotide binding
AT1G31600	RNA-binding (RRM/RBD/RNP motifs) family protein	c 1.9, n 3.7	Leaves	10.1093/nar/gkr40 6	At1G78190, Multifunctional methyltransferase subunit TRM112-like protein; Acts as an activator of both RNA and protein methyltransferases

IATE efflux family protein;
RNA-decapping enzyme subunit 2
ets as an activator of both RNA and protein
ises
tative plastid RNA methyltransferase;
/HIRLY 3, plastid, DNA binding
ibosomal protein L11 methyltransferase-related;
6S rRNA (cytidine1402-2'-O)-methyltransferase
d AT1G21580; RNA recognition motif (RRM)-
ein; Its function is described as RNA binding,
ing, zinc ion binding, nucleic acid binding;
IP37;
ITA
-adenosylmethionine synthetase 2
EA(D/H)-box RNA helicase family protein;
NA methyltransferase family protein;
-loop containing nucleoside triphosphate hydrolases
otein. Its function is described as helicase activity
NA methyltransferase family protein;
-adenosylmethionine synthetase family protein

AT1G78010	tRNA modification GTPase MnME	c 15.2, m 10.4	Leaves	10.3389/fpls.2014 .00678	AT2G13440, Involved in tRNA processing; AT1G51310, tRNA (5-methylaminomethyl-2-thiouridylate)- methyltransferases
AT2G13440	tRNA uridine 5- carboxymethylamino methyl modification enzyme MnmG- related	c 21.4; m 8.7	Leaves	10.1074/jbc.M308 435200	AT1G78010, tRNA modification GTPase; AT1G51310, tRNA (5- methylaminomethyl-2-thiouridylate)-methyltransferase activity
AT2G17970	RNA demethylase ALKBH9B/ALKBH 10B-like	n 9.1	Leaves, flowers, meristem	10.3389/fpls.2021 .701683	AT4G09980, Methyltransferase MT-A70 family protein; FIP37; AT4G35910, Plays a central role in 2-thiolation of mcm(5)S(2)U at tRNA wobble positions of tRNAs
AT2G19870	tRNA/rRNA methyltransferase (SpoU) family protein	c 13.6, m 11.2	Nd	/	AT4G25730, Adomet-dependent rrna methyltransferase spb1; AT1G54310, S-adenosyl-L-methionine-dependent methyltransferases superfamily protein; Its function is described as RNA binding; AT5G18570, Functions in the biogenesis of thylakoid membrane and plastid ribosome during chloroplast development; AT4G25730, Probable methyltransferase involved in the maturation of rRNA and in the biogenesis of ribosomal subunits
AT2G22090	RNA-binding (RRM/RBD/RNP motifs) family protein	c 7.7; n 7.0	Leaves, seeds, flowers	10.3390/biom100 40661	AT3G56860, Heterogeneous nuclear ribonucleoprotein (hnRNP)-like protein that acts as component of a complex regulating the turnover of mRNAs in the nucleus; AT5G64270, RNA binding splicing factor, putative; AT2G38610, RNA-binding KH domain-containing protein; AT4G11420, RNA-binding component of the eukaryotic translation initiation factor 3
AT1G32360	Zinc finger (CCCH- type) family protein of the m6A writer complex, interacts with HAKAI	n 8.5	flowers, seeds, leaves	10.3390/ijms2303 1572	AT1G08370 and AT5G13570, mRNA-decapping enzyme-like proteins; AT5G42810, Inositol-pentakisphosphate 2-kinase
AT2G39670	Ribosomal RNA large subunit methyltransferase RlmN/Cfr	c 21.2, m 3.0	Leaves, meristem	/	AT1G12800, RNA-binding, OB-fold-like protein; AT2G39670, RNA methyltransferase activity; Involved in rRNA processing; several chloroplast ribosomal RNAs

AT2G41040	S-adenosyl-L- methionine- dependent methyltransferases superfamily protein	c 31.2	Flowers	/	AT5g03900 and AT1g16080, Uncharacterized proteins; AT1G12250, uncharacterized pentapeptide repeat-containing protein; AT5G13650, putative chloroplastic elongation factor involved in response to chilling stress.
AT3G13060	contains YTH (YT521-B homology), an evolutionarily conserved m ⁶ A- dependent RNA binding domain	c 2.7; n 5.5	Leaves	10.3390/biom100 40661	AT1G02080, Transcription regulator; AT4G00660, DEAD-box ATP-dependent RNA helicase; AT3G27700, RNA recognition motif (RRM)-containing protein; AT1G16210, Uncharacterized protein
AT3G13180	RNA (C5-cytosine) methyltransferases	c 20.9; m 6.9	Leaves	10.1186/s12870- 017-1206-0	AT3G13180, NOL1/NOP2/sun family protein / antitermination NusB domain-containing protein; Its function is described as RNA binding; AT5G15550, Transducin/WD40 repeat-like superfamily protein; Required for maturation of ribosomal RNAs; AT5G04600, RNA-binding (RRM/RBD/RNP motifs) family protein
AT3G13460	The YTH (YT521-B homology) domain has been suggested to be an evolutionarily conserved m6A- dependent RNA binding domain	c 1.3; n 3.6	Meristem, seeds, leaves	10.7554/eLife.723 75	AT2G25090, CIPK, CBL-interacting serine/threonine-protein kinase 16; AT5G21140, Uncharacterized protein; AT4G38600, HEAT repeat, HECT-domain (ubiquitin-transferase)
AT3G21300	(Uracil-5)- methyltransferase family	c 6.3; m 13.3	Leaves	10.3390/biom100 40661	AT3G15460 and AT1G52930, Ribosomal rna processing brix domain protein; AT3G13230, RNA-binding KH domain-containing protein
AT3G23830	Glycine-rich RNA- bindingzZinc finger (CCCH-type)protein, induced by cold	c 11.8; m 8.5	Meristem	10.1007/s10930- 013-9504-3	AT3G03920, Putative H/ACA ribonucleoprotein complex; AT1G52930, Ribosomal rna processing brix domain protein; AT4G29510, Methylates (mono and asymmetric dimethylation) the guanidino nitrogens; AT4G12600, RNA binding ribosomal protein L7Ae; AT1G52930, Ribosomal rna processing brix domain protein
AT3G28460	RsmD, chloroplast RNA methyltransferase	c 22.9	Leaves	10.1093/pcp/pcab 060	AT4G26370, Antitermination NusB domain-containing protein; Its function is described as RNA binding;

					AT4G39040, RNA-binding CRS1 / YhbY (CRM) domain protein; Its function is described as RNA binding; AT5G46420, 16S rRNA processing and ribosome binding protein RimM family
	tRNA methyltransferase,	c 15.8	Leaves	10.1371/journal.p one.0242737	AT5G24840, tRNA (guanine-N(7)-)-methyltransferase; AT5G13830, FtsJ-like methyltransferase family protein;
AT3G56330	Trm1				AT1G15440, Periodic tryptophan protein 2; Involved in nucleolar processing of pre-18S ribosomal RNA;
					AT5G14520, Pescadillo-like protein; Required for maturation of ribosomal RNAs and formation of the large ribosomal subunit
	Trm13 is a tRNA methylase that		Leaves,	10.1186/s12870-	AT4G17610, tRNA/rRNA methyltransferase (SpoU) family protein;
AT4G01880	catalyzes 2'-O- methyladenosine	c 4.2; m 5.6	flowers	017-1206-0	AT3G26410, nucleic acid binding methyltransferases; AT5G01230, S-adenosyl-L-methionine-dependent 2'-O-ribose
	(Am) nucleoside formation on				methyltransferases superfamily; AT1G36310, S-adenosyl-L-methionine-dependent methyltransferases
	tRNA(Gly)(GCC) putative DOXA class				AT4G35910, Adenine nucleotide alpha hydrolases-like superfamily
AT4G02940	oxidative mRNA demethylase	c 7.0; m 3.7; n 3.3	Leaves, flowers	10.1186/s13059- 022-02814-8	protein; AT3G54170, FIP37;
	(AtALKBH10b)		т	10 1000/1547(00	AT4G09980; m6A methyltransferase MTA
AT4G04880	N6-mAMP deaminase (ADAL, renamed MAPDA)	Nd	Leaves, flowers	10.1080/1547628 6.2019.1642712	AT3G26410, tRNA methyltransferase activity; AT4G18905, Transducin/WD40 repeat-like superfamily protein, its function is described as nucleotide binding
AT4G24770	31-kDa RNA binding protein	c 26.9	Leaves	10.1093/jxb/erab1 65	AT1G78630, 50S ribosomal protein L13, chloroplastic; AT4G26370, Antitermination NusB domain-containing protein, its function is described as RNA binding; AT3G12930, Protein Iojap, chloroplastic; May be a ribosome
					silencing factor; AT3G20930, RNA-binding (rrm/rbd/rnp motifs) family protein
AT4G34110	PAB poly(A) binding protein 2	c 5.7	Leaves, flowers, meristem	10.1186/s13059- 019-1799-8	AT1G18070, Translation elongation factor EF1A; AT1G54170, Polyadenylate-binding protein-interacting protein 3; AT4G00660, DEAD-box ATP-dependent RNA helicase 8; AT5G44200, Nuclear cap-binding protein subunit 2

AT4G38020	tRNA/rRNA methyltransferase (SpoU) family protein	c 9.3; m 3.4	Leaves	/	AT1G54310, S-adenosyl-L-methionine-dependent methyltransferases; AT1G35680, 50S ribosomal protein L21 and L3-1; AT3G04820, Pseudouridine synthase; AT3G25920, 50S ribosomal protein L15; AT2G41670, P-loop containing nucleoside triphosphate hydrolase
AT5G10620	RNA methyltransferase RlmH	c 19.5	Leaves, flowers	/	AT5G53920, Ribosomal protei n L11 methyltransferase-related; AT1G45110, 16S rRNA (cytidine1402-2'-O)-methyltransferase; AT5G50110, S-adenosyl-L-methionine-dependent RNA methyltransferases; AT5G10910, S-adenosyl-L-methionine-dependent methyltransferase, MraW; AT5G24840, tRNA (guanine-N(7)-)-methyltransferase
AT5G10910	CMAL, chloroplast mraW m4C methylase family protein	13.1; m 11.6	Leaves, meristem	10.1093/nar/gkaa 129	AT1G64600, Methyltransferases;copper ion binding, contains Ribosomal protein Rsm22; AT1G49400, Uncharacterized protein; AT2G15820, Pentatricopeptide repeat-containing protein; AT1G79850, Plastid ribosomal small subunit protein 17; AT5G64580, Required for plastid development during embryogenesis
AT5G11530	Zinc finger (CCCH- type) family protein	n 7.8	Seeds	10.1073/pnas.192 0621117	AT5G64360, Chaperone DnaJ-domain superfamily protein, its function is described as heat shock protein binding; AT5G58230, Transducin/WD40 repeat-like superfamily protein
AT5G15390	tRNA/rRNA methyltransferase (SpoU) family protein	c 19.6; m 3.2	Leaves	/	AT1G54310, S-adenosyl-L-methionine-dependent methyltransferase; AT4G10650, P-loop containing nucleoside triphosphate hydrolases superfamily protein; AT5G26180, S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
AT5G17660	tRNA (guanine-N-7) methyltransferase	c 24.1; n 2.2	Leaves, flowers	/	AT5G14600, tRNA (adenine-N1-)-methyltransferase; AT2G22400, RNA methyltransferase; AT5G15810, tRNA (guanine(26)-N(2))-dimethyltransferase 1
AT5G26180	S-adenosyl-L- methionine- dependent methyltransferases superfamily protein	c 7.5; m 6.6	Meristem, leaves	10.3390/biom100 40661	AT3G16810, Pumilio (APUM) proteins containing PUF domain (eight repeats of approximately 36 amino acids each). PUF proteins regulate both mRNA stability and translation through sequence- specific binding to the 3' UTR of target mRNA transcripts; AT5G04600, RNA-binding (RRM/RBD/RNP motifs) family protein;

					AT2G40360, Transducin/WD40 repeat-like required for maturation of ribosomal RNAs; AT3G22660, Probable rRNA-processing protein EBP2 homolog
AT5G50110	Ribosomal RNA small subunit methyltransferase G [rsmG]	c 10.3; m 8.9	Leaves, flowers, meristem	/	AT1G78010, tRNA modification GTPase; AT3G20557, Hypothetical protein; Involved in the regulation of plant growth
AT5G58190	YTH (YT521-B homology), evolutionarily conserved m6A- dependent RNA binding domain	c 10.3; n 6.6	Leaves, flowers, meristem	10.3390/biom100 40661	AT1G02080, transcription regulator activity; AT2G27100, cap-binding complex (CBC) and both the pre-mRNA splicing and primary microRNAs (miRNAs) processing machinery; AT1G54440, Polynucleotidyl transferase, ribonuclease h fold protein
AT5G61020	ECT3 - Evolutionarily conserved C-terminal region 3 (ECT3); YTH domain	n 6.8	Meristem, flowers	10.1080/1559232 4.2022.2079308	AT3G27700, RNA recognition motif (RRM)-containing protein; AT2G39580, Putative zinc-finger domain; AT5G14520, NOP56-like pre RNA processing ribonucleoprotein
AT5G66360	Ribosomal RNA adenine dimethylase family protein	m 11.9; n 9.2; c 1.6	Petioles	10.1105/tpc.10.5. 699	AT3G13230, RNA-binding KH domain-containing protein, its function is described as RNA binding; AT4G26600, RNA methyltransferase activity

Supplemental table S2. List of oligonucleotides used in this study. Sequences are shown in 5' \rightarrow 3' direction

NAME	Sequence	Purpose
ACTIN_FWD	CTTGCACCAAGCAGCATGAA	qRT PCR
ACTIN_REV	CCGATCCAGACACTGTACTTCCTT	qRT PCR
ZAT12_FWD	AAGAAGCCTAACAACGACGC	qRT PCR
ZAT12_REV	AACAAAGCGCGTGTAACCAA	qRT PCR
ZAT10_FWD	CACAAGGCAAGCCACCGTAAG	qRT PCR
ZAT10_REV	TTGTCGCCGACGAGGTTGAATG	qRT PCR
SOD_FWD	CTGGTCCACATTTCAACCCC	qRT PCR
SOD_REV	CTTTCCGAGGTCATCAGGGT	qRT PCR
APX1_FWD	TAGGTCTGGCTTCGAAGGTG	qRT PCR
APX1_REV	CAGCAGCGTATTTCTCGACC	qRT PCR
CBF2_FWD	GGCTCCGATTACGAGTCTC	Probe for RNA gel blo
CBF2_REV	GCTCCATAAGGACACGTCATC	Probe for RNA gel blo
RD29A_FWD	CACACCAGCAGCACCCA	Probe for RNA gel blo
RD29A_REV	CCGAGAACAGAGTCAAAGTCC	Probe for RNA gel blo
COR15A_FWD	GGCGATGTCTTTCTCAGGAG	Probe for RNA gel blo
COR15A_REV	GTGGCATCCTTAGCCTCTC	Probe for RNA gel blo
MTA PROBE_FWD	ATGGAAACTGAATCTGATGACGC	Probe for RNA gel blo
MTA PROBE_REV	CTCTTTGCTATGGTTGAGCC	Probe for RNA gel blo
MTB PROBE_FWD	GAGCATCAAGATCGTGATTCC	Probe for RNA gel blo
MTB PROBE_REV	CCAGGAGGTCCACCACC	Probe for RNA gel blo
HAKAI PROBE_FWD	GAGGGATTCCCCGACGG	Probe for RNA gel blo
HAKAI PROBE_REV	GACCGTCTCTACCCCGG	Probe for RNA gel blo
PSAD_FWD	CCCAAATCCCTCTCCTTCAC	Probe for RNA gel blo
PSAD_REV	CCTTCTCTTCCTGGATTCGC	Probe for RNA gel blo
 PSAL_FWD	CAAAATTCATATCCCTGAGAGCCACAAC	Probe for RNA gel blo
 PSAL_REV	TTGACGAAGTAAGGAAGGTCAAGAACG	Probe for RNA gel blo
 PETC_FWD	ATGGCGTCCTCATCCCTTTCC	Probe for RNA gel blo
 PETC_REV	GACCACCATGGAGCATCACCA	Probe for RNA gel blo
ACTIN PROBE_FWD	ATGGCTGATGGTGAAGATATTC	Probe for RNA gel blo
ACTIN PROBE_REV	CGGTGAACAATCGACGGG	Probe for RNA gel blo
PSAA_80-MER	CCACATCTCCATTCAGGATTTCTTGGCCCACT	Probe for RNA gel blo
-	ATTGGCCAAACCACCTGAGCACTAGGTCCA	0
	ATGTGAGTAGGATCACTC	
PSAO 80-MER	ATCTCTCCTCAACCAGTTCCTCTCAAAGCA	Probe for RNA gel blo
_	AGTGACTCTTCCTCCCGATGCACCGGCGAGTCT	6
	CAAAGGGTTCTTGGCGC	
PSBO_80-MER	CGTACGTTAGCCTCTTTGGTGCTCCC	Probe for RNA gel blo
	TCCGCACCGGCCCCCGAGACAACGAGA	
	GCAGAGGTGGCTAGAGCAAAACCGGCG	

PETB_80-MER	ACTCATATTCCGGAAATATACAATGCA GAAAAAAATTTCGCGGTCGAACTACCA	Probe for RNA gel blot
	AAGGAGAATAGGCTAAAATTGTTAGA	
PSBA_80-MER	GTTTCCCTGATCAAACTAGAAGTTACC	Probe for RNA gel blot
	AAGGAACCATGCATAGCACTAAAAAGGGAG	
	CCGCCGAATACACCAGCTACACC	