



Article

Introducing *MdTFL1* Promotes Heading Date and Produces High-Throughput Phenotyping in Rice

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Supplementary Data

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Table S1. Sequences of primers used for gene cloning and genomic DNA PCR

Gene	Description		Primer sequence (5'-3')	Note
<i>MdTFL1</i>	TFL1-like protein	F	GGGGTACCATGAAAAGAGCCTCGGAG	Amplified the full length of target gene <i>MdTFL1</i>
		R	CGGGATCCCTAGCGTCTTCTAGCTG	
<i>hptII</i>	Hygromycin phosphotransferase	F	CTCGGAGGGCGAAGAATCTC	Amplified 563 bp of PCR product of <i>hptII</i> gene
		R	CAATGACCGCTGTTATGCGG	

Table S2. Sequences of primers used for RT-PCR and qRT-PCR

Gene	Description		Primer sequence (5'-3')	Accession No.
<i>MdTFL1</i>	TFL1-like protein	F	CTCGGAGGGCGAAGAATCTC	AB052994.1
		R	CAATGACCGCTGTTATGCGG	
<i>OsUbi1</i>	Polyubiquitin1	F	CACGGTTCAACAACATCCAG	NC_029261.1
		R	TGAAGACCCTGACTGGGAAG	
<i>OsEHD1</i>	EARLY HEADING DATE 1	F	TGGAGTATGGATCGCCCATT	NC_029265.1
		R	GCAGTTGCGGTTTCTAGGGT	
<i>OsHD1</i>	HEADING DATE 1	F	CACAATGCTGAGTGAGCAGC	NC_029261.1
		R	AGATGAACTCACGCTGTTGC	
<i>OsHD3B</i>	HEADING DATE 3B-like	F	GAATGGCCAGGTGCATTGGA	NC_029261.1
		R	CGCTTCCTACCAATTAGCGTG	
<i>RFT</i>	FLOWERING LOCUS T	F	CTGGTTTGCTTGGACTTGGC	NC_029264.1
		R	AGCTATGCCCTAGGTCCTCT	
<i>OsGhd7</i>	Transcription factor GHD7-like	F	TCCGTTTCATGTCGATGGGAC	NC_029262.1
		R	CGGGAAGACGAAGGGGAATC	
<i>OsMADS14</i>	MADS-box transcription factor 14-like	F	AGCGCTCATAACGTTCAAGGA	NC_029258.1
		R	TGTGCATTTGTTGTTGAGATGGA	
<i>OsMADS18</i>	MADS-box transcription factor 18-like	F	CAC TAACCGGGAGGAGCAAA	NC_029262.1
		R	GGTGAGTG CAGGGGCAAAAT	
<i>OsMADS50</i>	MADS-box transcription factor 50-like	F	TTATCCCAATTGGAGGGGCG	NC_029258.1
		R	ACCACCCTCCTTGCCTTTT	
<i>Gn1a/ OsCKX2</i>	Grain number 1a/ Cytokinin oxidase/dehydrogenase	F	GAGGTGTTCTACACGGTGGG	AB205193.1
		R	GCAGGTACTGCTTGTAGGCT	
<i>OsEXPA3</i>	Alpha-expansin-3	F	GGCAGAGCCTGTCCTTCATT	NM_001420295.1
		R	CAGA ACTGCACCCAAGAGGT	
<i>OsEXPA4</i>	Expansin-A4-like	F	ATAGCACGGTGTCTTCTTTCG	NM_001420547.1
		R	AGCCCAAACCTCTTCTCCAC	
<i>OsGA20ox3</i>	Gibberellin 20 oxidase 3	F	AGTATCGCACCGT CACGTTTC	AK060318.1
		R	GCCGCTGT CAGGATCAGATT	
<i>OsCPS2</i>	ent-copalyl diphosphate synthase 2-like	F	CGGCAATGCTTCTAACAGCC	AB066271.1
		R	CGATTGTGCGAACTAGCAGC	
<i>OsHD3A</i>	HEADING DATE 3A-like	F	CTAGCGCACTTGT TTTTCATGC	NC_029261.1
		R	GACGCTGCAGTAGTACCAGG	
<i>OsKO1</i>	ent-kaurene oxidase 1-like protein	F	CGGAAGAAGCCCCAATCCTT	NC_029261.1
		R	TTTATTGT CATGCAGCTGTTCC	
<i>OsKO2</i>	ent-kaurene oxidase 2-like protein	F	CTACAGAAGAAGCCCCGGTT	NC_029261.1
		R	TTCATTGT CATGCAGCTGTTCC	

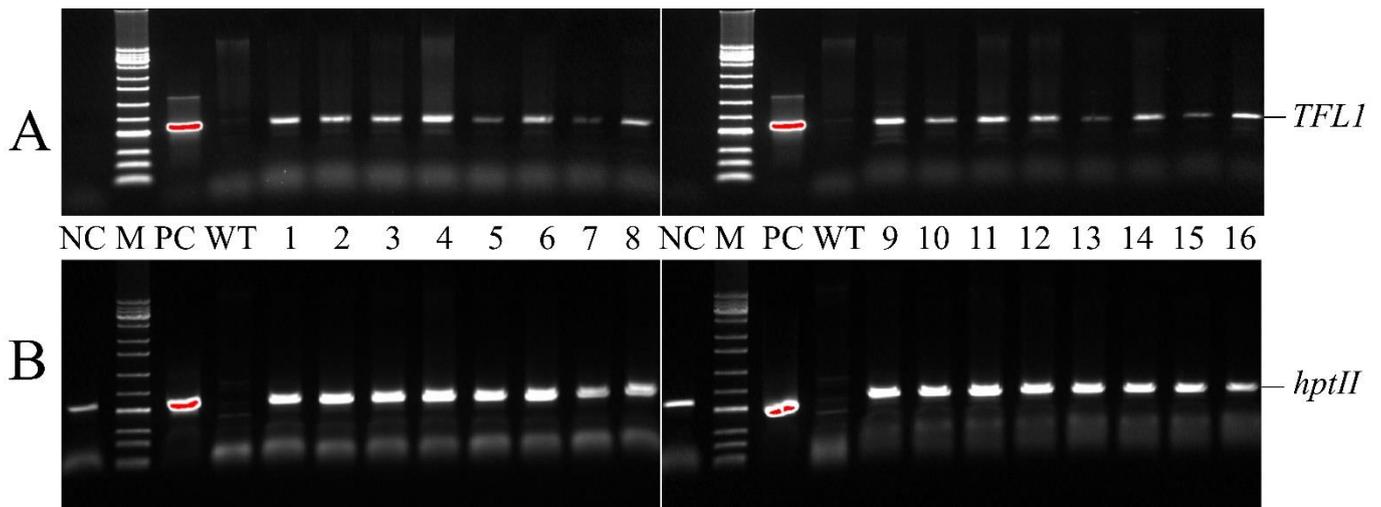


Figure S1. Genomic DNA PCR from rice callus. Detection of the target gene (*MdTFL1*) (A) and selection marker hygromycin phosphotransferase (*hptII*) (B) in transgenic rice callus. Lane PC, plasmid DNA of 3D::MdTFL1 used as positive controls; lane NC, genomic DNA of transgenic rice callus transformed with vector pMYD319 used as a mock; lane WT, genomic DNA of wild-type (non-transgenic callus) used as a negative control; lanes 1-16, independent 3D::MdTFL1 transgenic callus lines; lane M, 1 Kb plus DNA ladder marker.

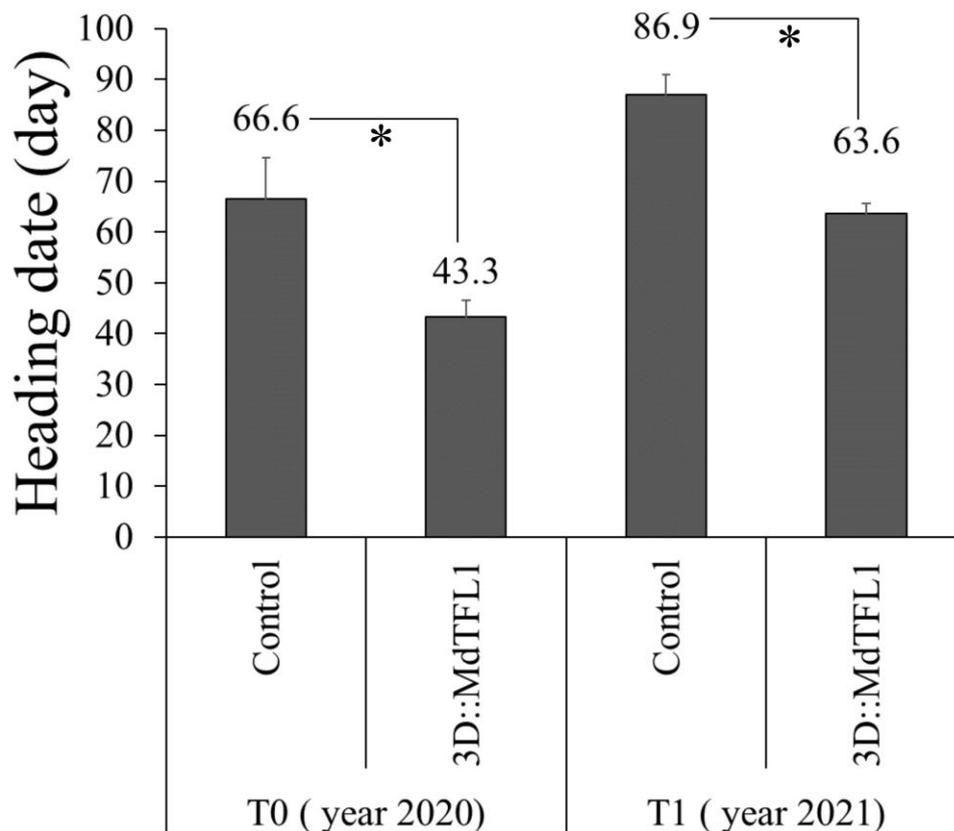


Figure S2. Heading date of T0 (the year 2020) and T1 (the year 2021) plants. The heading date of T0 plants was evaluated when the first panicle emerged after the in vitro seedlings were transferred to the soil. T1 plants were germinated from the seed of T0 plants, and their heading date was evaluated when the first panicle emerged after T1 seed sowing. Data are presented as mean \pm SD ($n \geq 20$). The asterisks indicate significant differences compared to control (WT and mock) ($p < 0.05$).

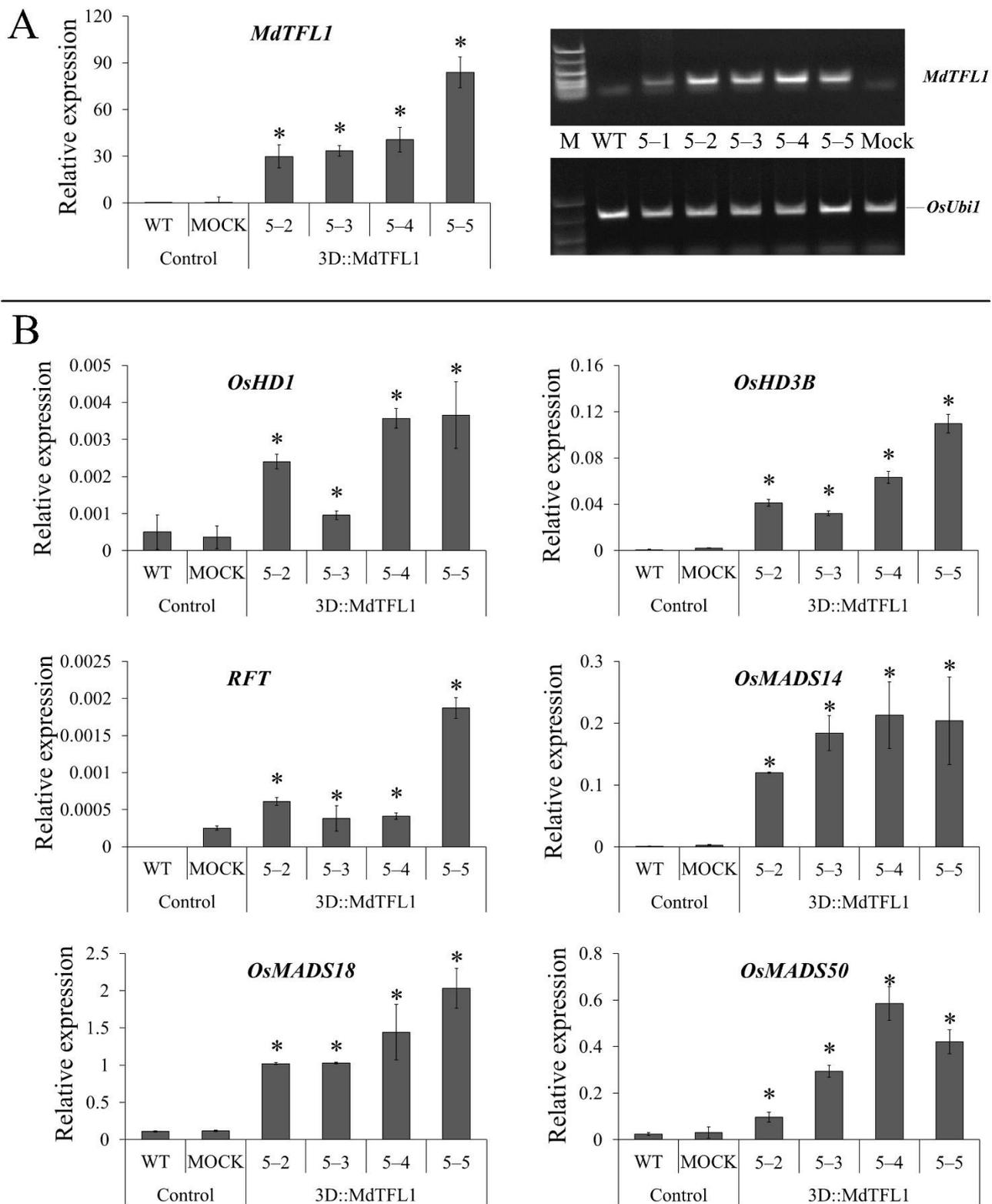


Figure S3. Expression profile of endogenous-flowering-related genes in rice callus. The expression level of *MdTFL1* (A) and rice flowering-related genes (B) was normalized to *OsUbi1*. Data represent the mean \pm SD from three biological replicates. The asterisks indicate significant differences compared to control (WT and mock) ($p < 0.05$).

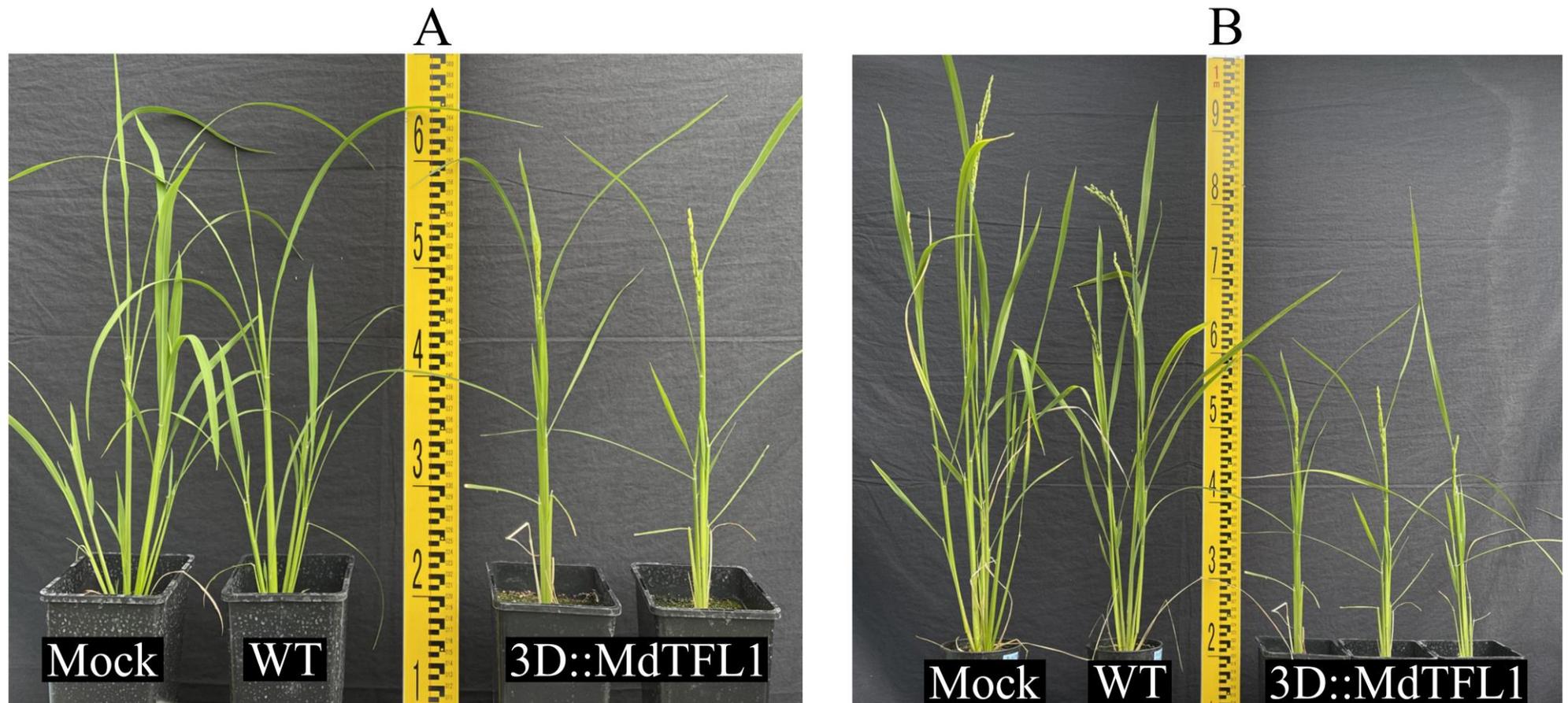


Figure S4. Phenotype of T0 plants (the year 2020) after transferring to the soil. (A) 3D::MdTFL1 transgenic rice plants showed early heading at 43 days after transferring the in vitro seedling to the soil while the control (mock and WT) plants remained at the vegetative stage. (B) Plant height of 3D::MdTFL1 transgenic rice plants was shorter than the control (mock and WT) plants at the same developmental stage (heading date).



Figure S5. Phenotype of T2 plants (the year 2022). 3D::MdTFL1 transgenic rice plants showed a high-throughput phenotype that exhibited a semi-draft with inclined leaf angles.

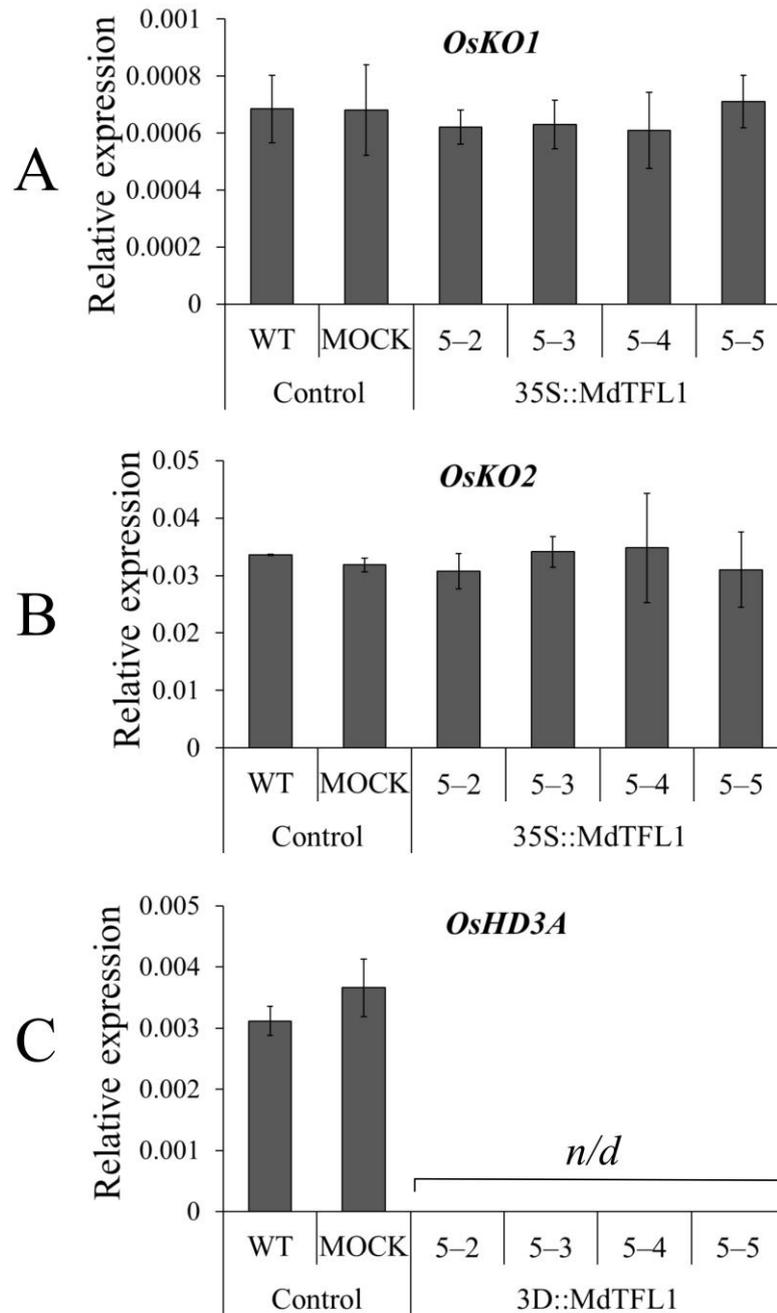


Figure S6. Expression profile of GA synthesis pathway and endogenous flowering-related genes and in rice plants. The expression level of *OsKO1* (A), *OsKO2* (B), and *OsHD3A* (A) was normalized to *OsUbi1*. Data represent the mean \pm SD from three biological replicates. *n/d* indicates *OsHD3A* was not detected in 35S::MdTFL1.