

Supplementary information

OsMYC2, an essential factor for JA-inductive sakuranetin production in rice, interacts with MYC2-like proteins that enhance its transactivation ability

Satoshi Ogawa¹, Koji Miyamoto², Keiichirou Nemoto³, Tatsuya Sawasaki³, Hisakazu Yamane², Hideaki Nojiri¹, and Kazunori Okada^{1,*}

Affiliation

¹Biotechnology Research Center, The University of Tokyo, Bunkyo-ku, Tokyo, Japan

²Department of Biosciences, Teikyo University, Utsunomiya, Tochigi, Japan

³Proteo-Science Center, Ehime University, Matsuyama, Ehime, Japan

*Corresponding author: E-mail, ukazokad@mail.ecc.u-tokyo.ac.jp; Fax: +81-35841-8030

Supplementary methods

Plant materials

See *Plant materials* in the main text.

Cloning

Standard methods for cloning were used for the construction. PCR-amplified DNA fragments were sequenced after cloning into vectors. The vectors referred to in the *supplementary methods* and primers used for the cloning are listed in Supplementary Tables S6 and S7, respectively.

pGL4-OsNOMT₂₅₀, *pGL4-OsNOMT₁₅₀*, and *pGL4-OsNOMT₅₀*: The 5' 250-, 150-, or 50-bp upstream region of the *OsnOMT* promoter, extending from the transcriptional start site, was PCR-amplified and cloned into the pZErO2 vector (Invitrogen, CA, USA). Each promoter region was extracted and inserted into the multi-cloning sites of pGL4.10-Tnos (see the main text).

pGL4-OsNOMT₂₅₀₋₂₆: The region -250 to -26 bp from the transcriptional start site of *OsnOMT* was PCR-amplified and inserted into *EcoRV*-digested pGL4.10-Tnos.

Luciferase activity assay

See *Luciferase activity assay* in the main text.

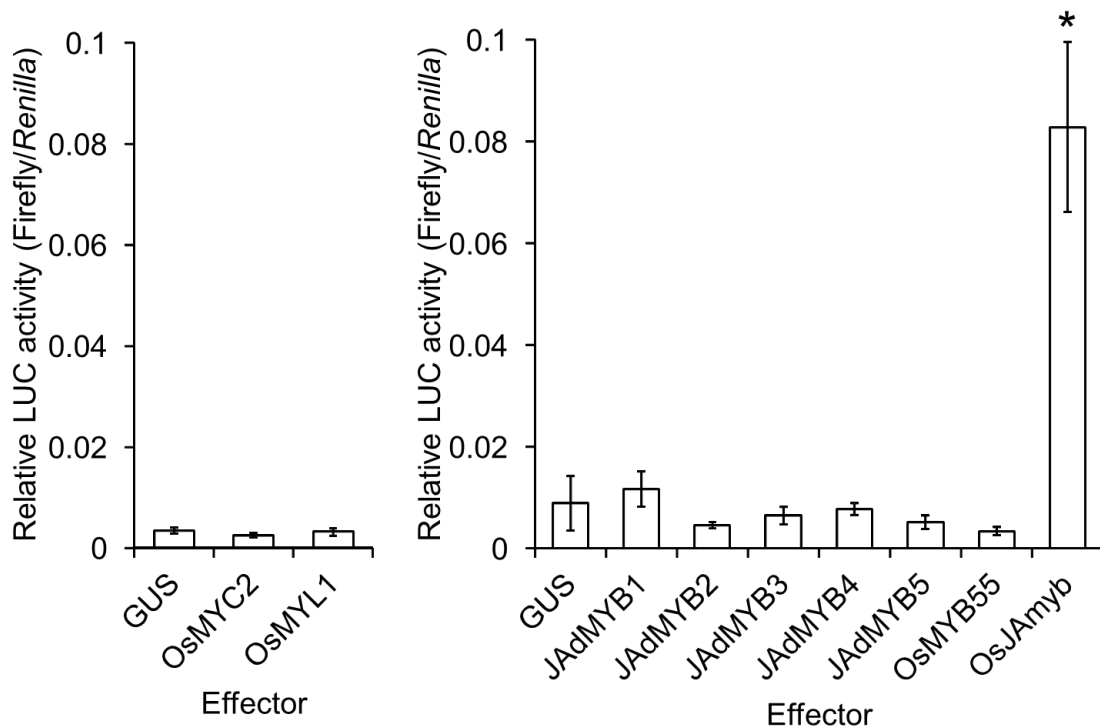
Optimized synthetic CDS of *OsMYC2*.

ATGAACCTCTGGACCGATGATAACGCTTCTATGATGGAAGCTTTTATGGCTTCTGCTGAT
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CATCATCACCAACAGCAGCAACAGCAAGTTCTTCCCTCCTCCTGCTGCTGCACCAGCTGCT
GCTGCTTTTAATCAGGATACTCTCCAGCAGAGGCTCCAGTCTATTATCGAGGGATCTAGA
GAGACTTGGACCTACGCTATCTTCTGGCAGTCATCTATCGATGTGTCTACCGGTGCTTCT
CTTCTCGGATGGGGTGATGGATATTACAAGGGATGTGATGATGATAAGAGGAAGCAGAGG
TCATCTACCCCTGCTGCAGCTGCTGAACAAGAGCATAGAAAGAGAGTGCTCAGGGAACTC
AACTCTCTTATCGCTGGTGCTGGTGCTGCTCCTGATGAAGCTGTTGAAGAAGAGGTGACC
GATACCGAGTGGTTCTTCCCTTGTGTCTATGACCCAGTCTTTCCCTAACGGACTTGGACTT
CCTGGACAGGCTCTTTTGGCTGCTCAACCTACTTGGATCGCTACCGGACTTTCTTCTGCT
CCTTGCGATAGAGCTAGACAGGCTTACACTTTCGGACTCAGGACTATGGTTTGCCTTCCCT
CTCGCTACTGGTGTTCTCGAGCTTGGATCTACCGATGTGATCTTCCAGACCGGTGATTCT
ATCCCTAGAATCAGGGCTCTCTTCAACCTCAGTGCTGCAGCAGCTTCTTCTTGGCCTCCT
CATCCAGATGCTGCTTCAGCTGATCCTTCTGTTCTTTGGCTTGCTGATGCTCCTCCTATG
GATATGAAGGATTCTATCTCTGCTGCTGATATCTCTGTGTCTAAGCCTCCTCCTCCTCCA
CCTCATCAAATCCAGCATTTCGAGAACGGATCTACCTCTACCCTTACCGAGAACCCTTCT
CCTTCTGTGCATGCTCCTACACCTTCTCAACCTGCAGCTCCTCCACAAAGACAACAACAA
CAGCAACAATCTTCTCAGGCTCAGCAGGGACCTTTCAGAAGAGAGCTTAACTTCTCTGAT
TTCGCTTCTAACGGTGGTGCTGCTGCTCCACCATTTTTCAAGCCTGAGACTGGTGAGATC
CTCAACTTCGGAAACGATTCTTCATCTGGAAGAAGAAACCCTTACCAGCTCCACCAGCA
GCTACAGCTTCTCTTACTACTGCTCCTGGATCACTCTTCTCACAGCACACTCCTACTCTT
ACCGCTGCTGCTAACGATGCTAAGTCTAACAACCAGAAAAGATCTATGGAAGCTACCTCT
AGGGCTTCTAACACCAACAACCATCCTGCTGCAACCGCTAACGAGGGAATGCTCTCTTTT
TCATCTGCTCCAACCACCAGGCCTTCTACTGGAAGTGGTGCTCCTGCTAAGTCAGAGTCT
GATCACTCTGATCTCGAGGCTTCAGTGAGAGAGGTTGAGTCATCTAGAGTTGTGGCACCT
CCTCCAGAGGCTGAGAAAAGACCTAGAAAAAGAGGTAGGAAGCCTGCTAACGGAAGGGAA
GAACCTCTCAACCATGTGGAAGCTGAGAGGCAGAGAAGAGAGAAGCTCAACCAGAGATTC
TACGCTCTCAGAGCTGTGGTGCCTAACGTGTCAAAGATGGATAAGGCTTCACTCCTCGGA
GATGCTATCTCTTACATCAACGAGCTTAGGGGAAAGCTCACCGCTCTCGAGACTGATAAG
GAAACCTTGCAGTCTCAGATGGAATCTCTCAAGAAAGAAAGGGATGCTAGGCCTCCTGCT
CCTTCAGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGT
ATCCTTGGACTCGAGGCTATGATTAGAGTGCAGTGCCACAAGAGAAACCACCCAGCTGCT
AGACTTATGACCGCTCTTAGAGAACTCGATCTCGATGTGTACCACGCTTCTGTGTCTGTG
GTGAAGGATCTCATGATCCAGCAAGTGGCTGTGAAGATGGCTTCTAGGGTGTACTCTCAG
GATCAGTTGAACGCTGCTCTTACACCAGAATCGCTGAACCTGGTACTGCTGCTAGATGA

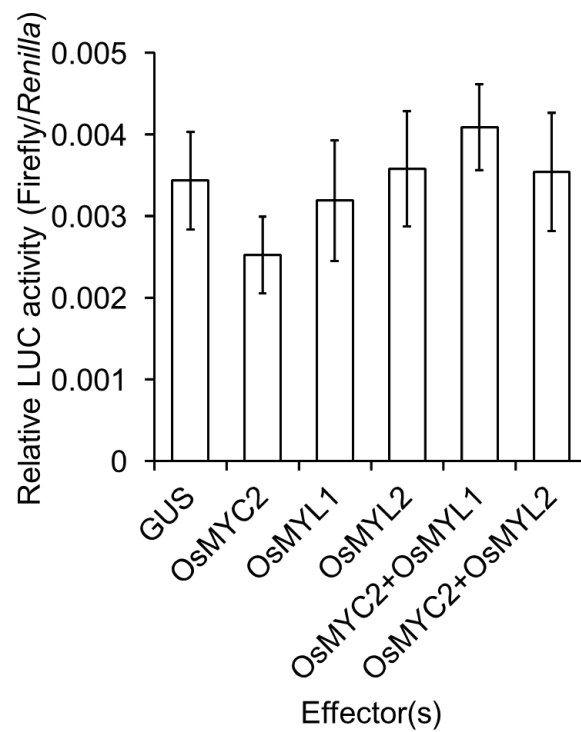
CDS of *OsMYL1* described in this article.

ATGTCGTGGTCCGAGACGGACGCCGCGCTGTTTCGCGGCGGTGCTGGGGCACGACGCGGCG
CATCACCTGGCCACCACGCCGCCGACCTCGACGCGCCGGAGGGTTCGCCGTTCGTCGGCC
GAGCTGCAGGCGAGCCTGCACGACCTCGTCGAGCGGCAGGGAGGGGCCTGGACGTACGGC
ATCTTCTGGCAGGAGTCCC GCGCGCCGGTGCCGCCAGCGGCCGCGCTGCCCGCGCCGTG
CTCGGCTGGGGCGACGGCCACTGCCGCGACGGTGCCGGACACGGGGAGGTTCGGCGCCGCG
GAGAGGAGCGTGCGCGGAAGCGCGTGCTGCTGCGGCTGCACGCGCTGTACGGAGGCGGG
GATGAGGATGGCGCCGACTACGCGCTCCGGCTGGACCGGGTCACCGGCGCCGAGATGTAC
TTCCTGGCGTCCATGTACTTCTCCTTCCC GAGGGCTCGGGCGGACCGGGCCGCGCTCTG
GCCTCCGGCCGCCACGCCTGGGCGGACGTAGACCCCCACCTTCCGGCTCCGGTAGCGCG
CCAGGGTGGTACGTTTCGCTCGTCTCTCGCCCAGTCCGCGGGGTTACGCACCGTTCGTCTTC
CTCCCGTGCAAGGGCGGCGTTCTCGAGCTCGGTTCCGTTCGTAGCCATCCGCGAGACCCCC
GAGGTCTTGCGCGCCATCCAATCTGCCATGCGCGCCGTGCCAGCTCCGCGGAAGATTTTC
ATGAGAATCTTCGGCAAGGATCTCTCACCCGGCCGACCGTCCCAGCCCATGGGATGCGAC
GCCCCATGGACGCCGCGGCTCGTTGTCCAAACCACGCCGGTGCGCCCAGCCAAGAAGGAG
GTGGTCAAGGCGAAACCAGCCGAGCCCCCAAGAGCTTGGACTTCTCCAAGGCGAACGTG
CAGGAACAGGCCGGCGGCCAGGAGCGGCGGCCGCGGAAGCGTGGGCGCAAGCCGGCGAAC
GGGCGGGAGGAGCCGCTGAACCACGTGGAGGCGGAGCGGCAGCGGGGAGAAGCTGAAC
CAGAGGTTCTACGCGCTGCGCGCGGTGGTGCCCAAGATCTCCAAGATGGACAAGGCGTCC
CTGCTCAGCGACGCCATCGCGTACATCCAGGAGCTGGAGGCCCGGCTCAGGGGCGACGCG
CCCGTGCCCGCGCGGGCGGATGGGCCGGCCGTGGAGGTGAAGGCAATGCAGGACGAGGTG
GTGCTGCGCGTGACCACGCCCTGGACGAGCACCCCATCTCCAGGGTGTTCACGCCATG
AGGGAATCCCAGATCAGCGTCGTGGCGTCGGACGTGGCGGTGTCGGACGACGCCGTCACG
CACACGCTCATGGTGCGGTCGGCCGGGCCGGAGCGGCTCACGGCGGAGACGGTGCTCGCG
GCGATGTCGCGGGGGGTGAGCGTCACCACTCCCTCCCCGTGA

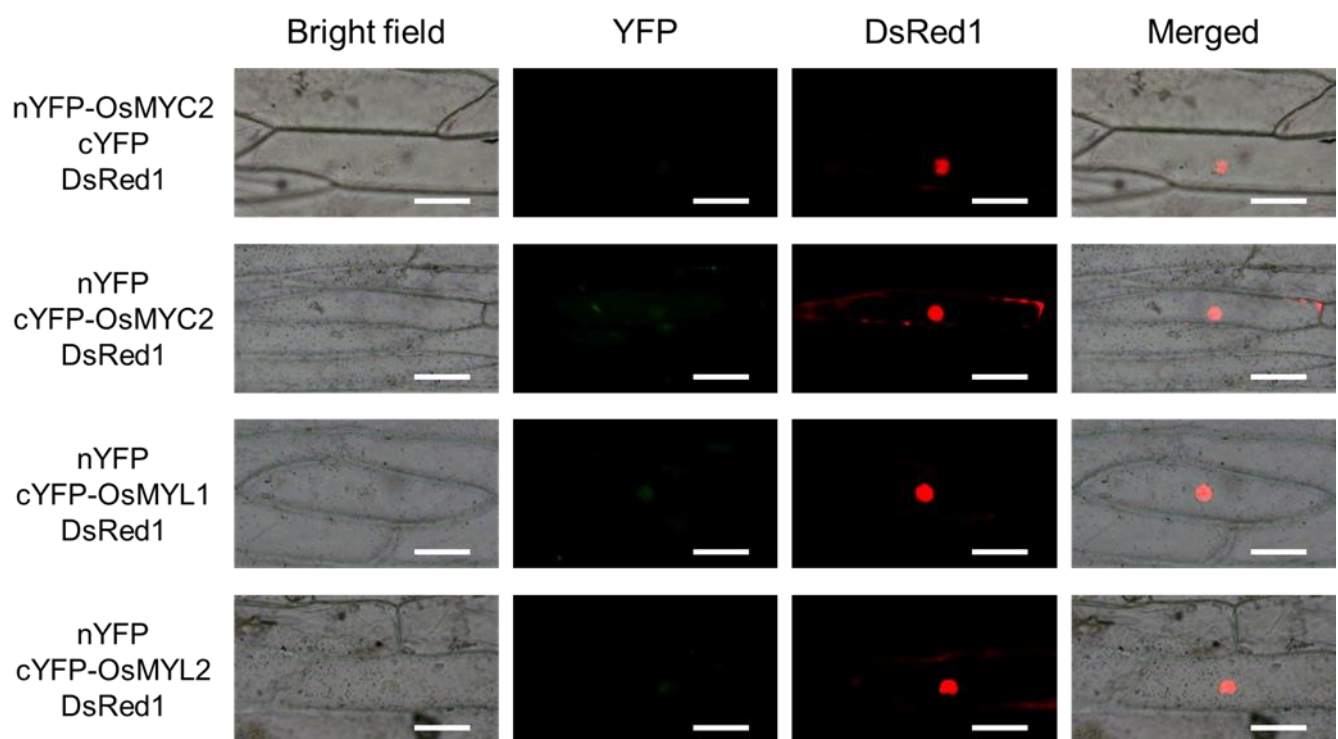
Underlined region is annotated as intron in RAP-DB, but not in MSU libraries.



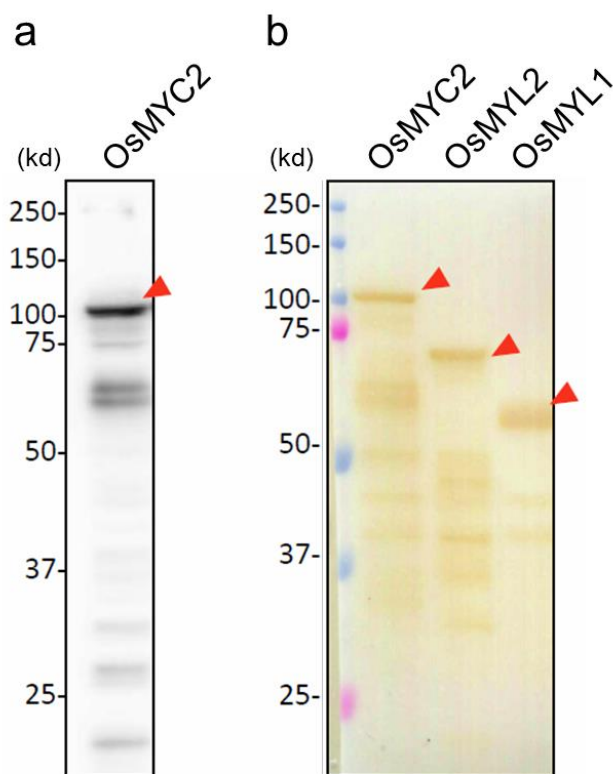
Supplementary Figure S1 ***OsNOMT* promoter-independent effects of JA-induced transcription factors.** Relative luciferase activities in the bombarded rice leaves. Data are presented as FLUC/RLUC \pm standard error. n = 3–5. *P < 0.05, compared to the data with GUS as the effector.



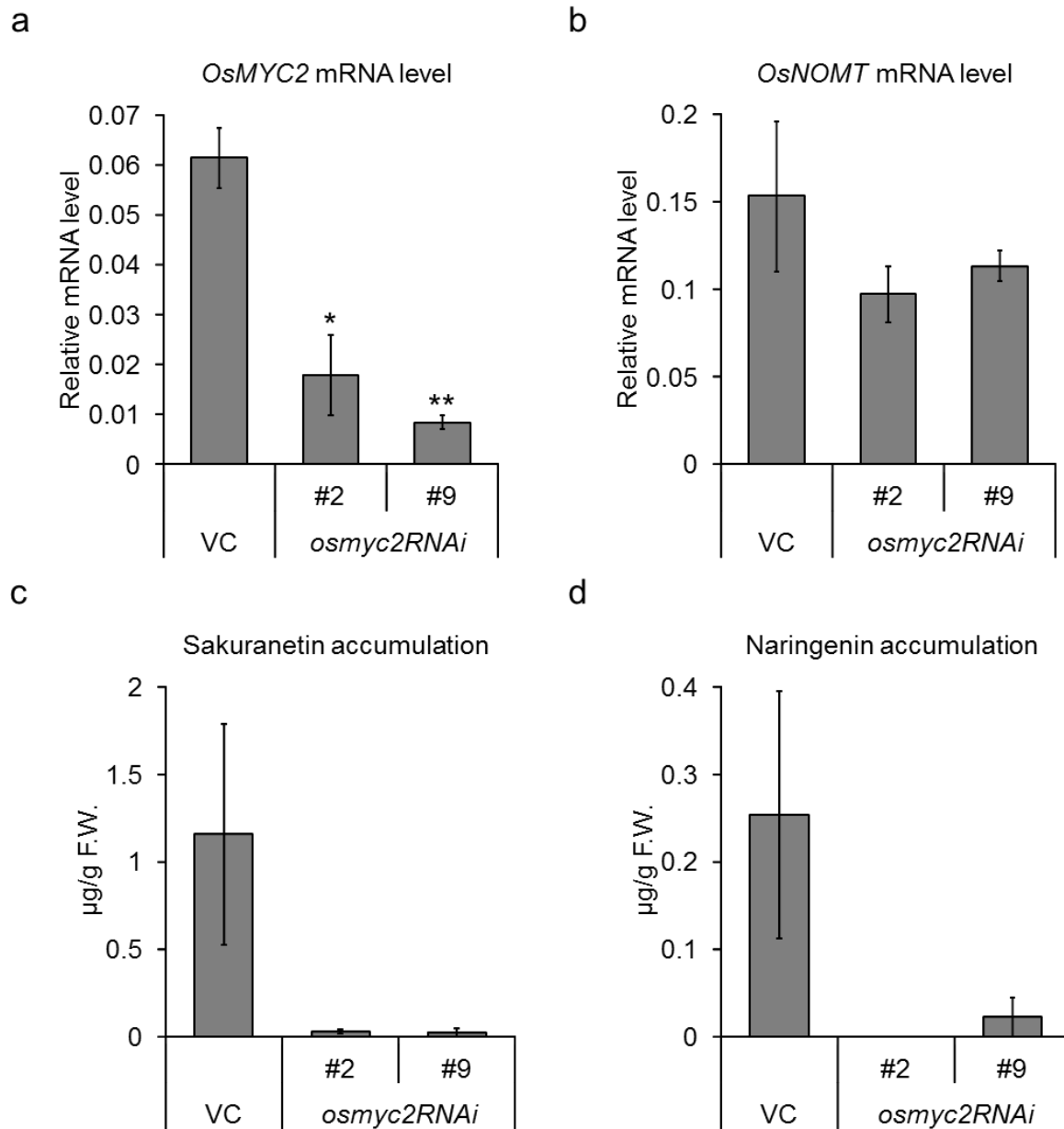
Supplementary Figure S2 *OsNOMT* promoter-independent effects of **OsMYC2**, **OsMYL1**, and **OsMYL2**. Relative luciferase activities in the bombarded rice leaves. Data are presented as FLUC/RLUC \pm standard error. n = 4.



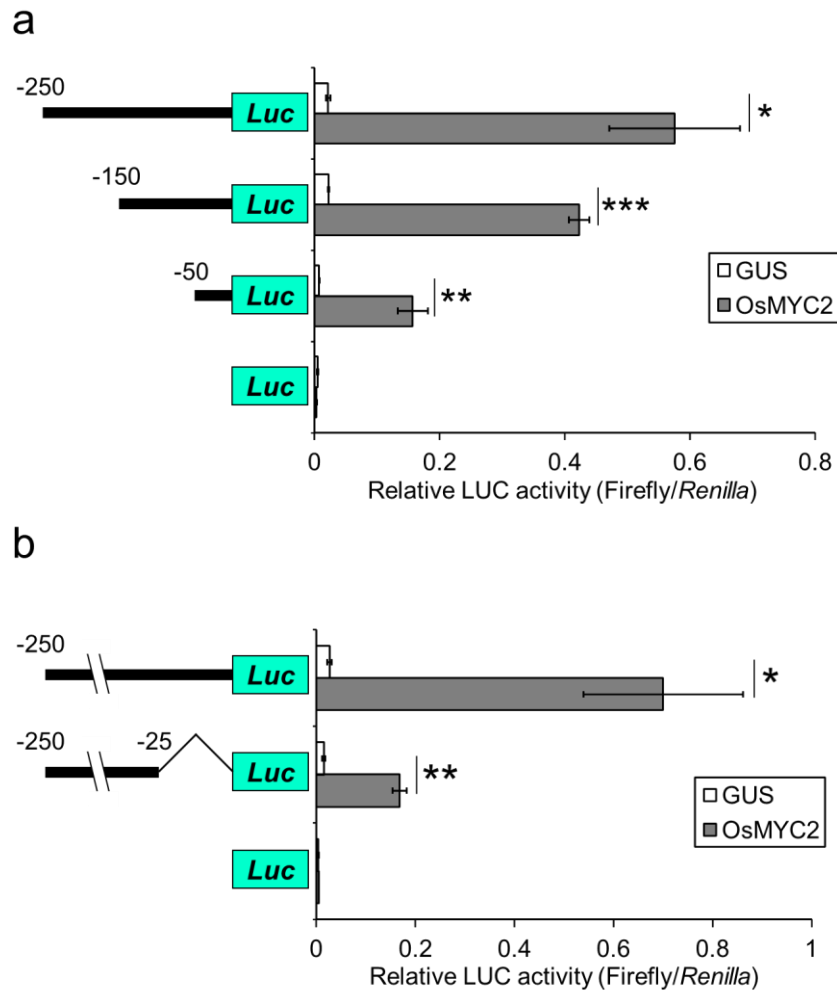
Supplementary Figure S3 **Negative controls of BiFC assays.** Bars = 100 μ m.



Supplementary Figure S4 **Recombinant proteins expressed using the wheat germ cell-free system.** Two micrograms of the crude protein was applied in each lane. Red arrows indicate the desired proteins. **(a)** Detection of biotinylated-OsMYC2 by using anti-Biotin HRP. **(b)** Detection of FLAG-tagged proteins by using anti-FLAG M2 HRP. A chemiluminescent reagent visualized the recombinant proteins without LAS detection.



Supplementary Figure S5 **Gene expression and phytoalexin accumulation in the CuCl₂-treated *OsMYC2*-knockdown plants (*osmyc2RNAi*).** (a, b) Expression levels of *OsMYC2* (a) and *OsNOMT* (b). Total RNA from the rice leaves after 6 h of 500 µM CuCl₂ treatment was extracted. qRT-PCR analysis was performed using the cDNA samples synthesized from the total RNA. (c, d) Contents of sakuranetin (c) and naringenin (d) in the rice leaves. Data are presented as mean ± standard error values. (a, b) n = 3–4, (c, d) n = 4–6. *P < 0.05, **P < 0.01, compared to the VC data.



Supplementary Figure S6 **Deletion assay for the *OsNOMT* promoter.** 5'-truncated series (**a**) and 3'-truncated series (**b**) were used for the assay. Data are presented as FLUC/RLUC \pm standard error. n = 4. *P < 0.05, **P < 0.01, ***P < 0.001.

Supplementary Table S1 **CuCl₂-induced expression of *OsNOMT* in transcriptome analysis.**

Gene ID	Gene name	WT CuCl ₂ 2 h		WT CuCl ₂ 6 h		WT CuCl ₂ 24 h	
		/WT water 2 h	Q value	/WT water 6 h	Q value	/WT water 24 h	Q value
Os12g0240900	OsNOMT	1.115	#	12.213	0.0038	17.002	0.0011

indicates that fold change is ≤ 2 and/or that FDR is ≥ 0.05 .

Supplementary Table S2 **JA-inducible transcriptional factors selected by transcriptome analysis using *cpm2*.**

Type	Gene ID	Gene name	Description	WT		<i>cpm2</i>		WT		<i>cpm2</i>	
				CuCl ₂ 2 h	Q value	CuCl ₂ 2 h/	Q value	CuCl ₂ 6 h	Q value	CuCl ₂ 6 h	Q value
				/WT	WT	/WT	WT	/WT	WT	/WT	WT
				water 2 h	Q value	CuCl ₂ 2 h	Q value	water 6 h	Q value	CuCl ₂ 6 h	Q value
HLH/bHLH	Os01g0108600		Basic helix-loop-helix dimerisation region bHLH domain containing protein. [Os01t0108600-01]	2.208	0.0031	0.081	0.0085	1.401	#	0.042	#
	Os01g0196300		Helix-loop-helix DNA-binding domain containing protein. [Os01t0196300-01]	65.337	0.0041	0.488	0.0204	130.220	#	2.781	#
	Os01g0705700	OsMYL1	Similar to Transcription factor ICE1 (Inducer of CBF expression 1) (Basic helix- loop-helix protein 116) (bHLH116) (AtbHLH116). [Os01t0705700-01]	70.452	0.0019	0.107	0.0015	49.453	0.0004	0.045	0.0054
	Os02g0120500		Helix-loop-helix DNA-binding domain containing protein. [Os02t0120500-02]	2.960	0.0337	0.327	0.0440	1.902	#	1.018	#
	Os02g0603600		Similar to Phytochrome-interacting factor 3 (Phytochrome-associated protein 3) (Basic helix-loop-helix protein 8) (bHLH8) (AtbHLH008). [Os02t0603600-01]	1.080	#	0.870	#	2.380	0.0080	0.401	0.0371
	Os03g0741100		Basic helix-loop-helix dimerisation region bHLH domain	38.999	0.0017	0.128	0.0183	22.264	#	0.229	#

			containing protein.								
			[Os03t0741100-01]								
Os04g0301500			Helix-loop-helix	631.858	0.0137	0.007	0.0066	58.968	#	0.016	#
			DNA-binding domain								
			containing protein.								
			[Os04t0301500-01]								
Os05g0163900			Helix-loop-helix	9.984	0.0137	0.092	0.0126	5.653	0.0009	0.344	0.0414
			DNA-binding domain								
			containing protein.								
			[Os05t0163900-01]								
Os07g0628500			Basic helix-loop-helix	1.398	#	0.483	#	3.197	0.0261	0.417	0.0210
			dimerisation region								
			bHLH domain								
			containing protein.								
			[Os07t0628500-01]								
Os09g0501600			Similar to MYC1.	1.901	#	0.417	#	6.826	0.0039	0.288	0.0035
			[Os09t0501600-01]								
Os10g0376900			Helix-loop-helix	2.558	#	0.243	#	3.365	0.0013	0.139	0.0251
			DNA-binding domain								
			containing protein.								
			[Os10t0376900-01]								
Os10g0575000	OsMYC2		Similar to Transcription	3.177	0.0083	0.177	0.0103	2.355	0.0011	0.214	0.0179
			factor MYC7E								
			(Fragment).								
			[Os10t0575000-01]								
MYB	Os01g0192300	JAdMYB3	Similar to R1MYB1	15.686	0.0072	0.083	0.0396	9.418	0.0330	0.104	0.0117
			protein.								
			[Os01t0192300-02]								
Os01g0702700	JAdMYB1		Similar to Transcription	2.210	0.0101	0.032	0.0065	3.497	0.0027	0.010	0.0098
			factor MYB86								
			(Myb-related protein 86)								
			(AtMYB86) (Myb								
			homolog 4) (AtMyb4).								
			[Os01t0702700-01]								
Os02g0641300			Rep: ATMYB4-like -	1.745	#	0.308	#	2.171	0.0063	0.319	0.0060
			Oryza sativa subsp.								
			japonica (Rice),								
			complete [TC417408]								

	Os05g0429900	JAdMYB4	Similar to MybHv5 (Fragment). [Os05t0429900-01]	10.548	0.0041	0.343	0.0016	11.496	0.0098	0.134	0.0009
	Os05g0442400		Similar to MybSt1. [Os05t0442400-01]	132.454	0.0128	0.014	0.0033	49.550	0.0306	0.046	0.0034
	Os05g0553400	OsMYB55	Similar to Myb-related transcription factor-like protein (MYB transcription factor). [Os05t0553400-01]	7.073	0.0025	0.409	0.0247	10.666	0.0025	0.194	0.0027
	Os08g0549000	JAdMYB5	Similar to MybHv5 (Fragment). [Os08t0549000-00]	4.410	0.0060	0.419	0.0123	5.257	0.0035	0.329	0.0191
	Os11g0684000	OsJAmyb	Similar to Transcription factor MYB21 (Myb-related protein 21) (AtMYB21) (Myb homolog 3) (AtMyb3). [Os11t0684000-01]	20.002	0.0008	0.126	0.0063	24.901	0.0004	0.070	0.0034
	Os12g0564100	JAdMYB2	Similar to R2R3MYB-domain protein (Fragment). [Os12t0564100-01]	4.645	0.0048	0.019	0.0037	2.439	0.0280	0.022	0.0203

bZIP	Os06g0662200		bZIP transcription factor, bZIP-1 domain containing protein. [Os06t0662200-01]	0.647	#	1.451	#	3.875	0.0070	0.079	0.0175
	Os08g0357300		Similar to BZIP DNA-binding protein. [Os08t0357300-01]	0.605	#	0.597	#	4.375	0.0007	0.195	0.0207
	Os12g0560900		Similar to BZIP protein. [Os12t0560900-01]	1.889	#	0.561	#	3.170	0.0027	0.443	0.0088

WRKY	Os01g0185900		Similar to WRKY 1 (Fragment). [Os01t0185900-01]	9.353	0.0009	0.413	0.0064	12.934	0.0008	0.466	0.0149
	Os01g0186000		Similar to WRKY transcription factor 10. [Os01t0186000-01]	24.393	0.0022	0.050	0.0159	26.186	0.0012	0.065	0.0057

Os01g0626400	DNA-binding protein WRKY2-like (WRKY transcription factor 11) (WRKY16). [Os01t0626400-01]	5.141	0.0009	0.185	0.0092	9.051	0.0014	0.154	0.0027
Os01g0714800	WRKY transcription factor 26. [Os01t0714800-01]	1478.633	0.0009	0.049	0.0075	1800.347	0.0003	0.363	0.0091
Os01g0734000	Similar to WRKY DNA binding protein. [Os01t0734000-01]	4.291	0.0013	0.225	0.0108	3.804	0.0073	0.124	0.0066
Os01g0750100	Similar to WRKY13 transcription factor. [Os01t0750100-03]	1.366	#	0.534	#	2.201	0.0033	0.320	0.0100
Os01g0821600	WRKY transcription factor 48-like protein (WRKY transcription factor 21). [Os01t0821600-01]	58.586	0.0003	0.485	0.0072	35.706	#	0.679	#
Os02g0770500	Similar to WRKY transcription factor 32. [Os02t0770500-00]	9.112	0.0009	0.196	0.0227	8.687	0.0020	0.149	0.0054
Os05g0583000	WRKY transcription factor 34. [Os05t0583000-01]	1.790	#	0.194	#	2.318	0.0033	0.136	0.0058
Os11g0117500	DNA-binding WRKY domain containing protein. [Os11t0117500-01]	398.079	0.0115	0.026	0.0015	731.906	0.0077	0.076	0.0109
Os11g0490900	Similar to WRKY transcription factor 72. [Os11t0490900-01]	29.821	0.0011	0.321	0.0035	39.339	#	0.967	#
Os12g0116700	Similar to WRKY transcription factor 64. [Os12t0116700-01]	345.859	0.0072	0.022	0.0021	582.052	0.0041	0.063	0.0041
NAC	Os01g0816100 Similar to NAC domain protein. [Os01t0816100-01]	140.804	0.0010	0.140	0.0047	199.857	0.0003	0.476	0.0231

Os03g0777000	Similar to NAC-domain containing protein 19 (ANAC019) (ANAC) (Abscisic-acid- responsive NAC). [Os03t0777000-01]	10.184	0.0007	0.165	0.0068	14.617	#	0.910	#		
Os03g0815100	Similar to OsNAC6 protein. [Os03t0815100-01]	8.674	0.0012	0.489	0.0412	9.519	#	0.583	#		
Os08g0200600	Similar to NAC-domain containing protein 21/22 (ANAC021) (ANAC022). Splice isoform 2. [Os08t0200600-01]	1.512	#	0.830	#	5.387	0.0003	0.125	0.0045		
Os11g0126900	Similar to NAC domain transcription factor. [Os11t0126900-01]	2.607	0.0180	0.310	0.0066	1.163	#	0.219	#		
(OsMYL2)	Os01g0235700	OsMYL2	Similar to BHLH transcription factor (Fragment).	1.027	#	1.004	#	1.156	#	0.971	#

indicates that fold change is not sufficient for selection and/or that FDR is ≥ 0.05 .

Supplementary Table S3 Vectors used in this study.

Vector	Relevant characteristics	Reference
pENTR/D-TOPO	Km ^R , Gateway system entry vector	Invitrogen
pENTR-RLUC	Km ^R , pENTR/D-TOPO containing <i>renilla luciferase</i> CDS	This study
pRL	Amp ^R , CaMV 35S promoter-Renilla <i>LUC</i>	Promega
pUbi-RfA-Tnos	Amp ^R , Cm ^R , <i>ccdB</i> , pUCAP/Ubi-NT containing gateway cassette (RfA, invitrogen) cloned into <i>Bam</i> HI/ <i>Sac</i> I sites after blunting	15
pUbi-RLUC	Amp ^R , pUbi-RfA-Tnos containing <i>renilla luciferase</i> CDS	This study
pZErO2	Km ^R , <i>ccdB</i> , cloning vector	Invitrogen
pZErO2-OsNOMT ₁₀₀₀	Km ^R pZErO2 containing 5' 1-kb upstream region of <i>OsNOMT</i>	This study
pGL4-Tnos	Amp ^R , Firefly <i>LUC-NOS</i> terminator	This study
pGL4-OsNOMT ₁₀₀₀	Amp ^R , 5' 1-kb upstream region of <i>OsNOMT</i> - Firefly <i>LUC-NOS</i> terminator	This study
pENTR-OsMYC2	Km ^R , pENTR/D-TOPO containing <i>OsMYC2</i> CDS	This study
pENTR-OsMYL1	Km ^R , pENTR/D-TOPO containing <i>OsMYL1</i> CDS	This study
pENTR-OsMYL2	Km ^R , pENTR/D-TOPO containing <i>OsMYL2</i> CDS	This study
pENTR-JAdMYB1	Km ^R , pENTR/D-TOPO containing <i>JA-dependent (JAd) MYB1</i> CDS	This study
pENTR-JAdMYB2	Km ^R , pENTR/D-TOPO containing <i>JAdMYB2</i> CDS	This study
pENTR-JAdMYB3	Km ^R , pENTR/D-TOPO containing <i>JAdMYB3</i> CDS	This study
pENTR-JAdMYB4	Km ^R , pENTR/D-TOPO containing <i>JAdMYB4</i> CDS	This study
pENTR-JAdMYB5	Km ^R , pENTR/D-TOPO containing <i>JAdMYB5</i> CDS	This study
pENTR-OsJAmyb	Km ^R , pENTR/D-TOPO containing <i>OsJAmyb</i> CDS	This study
pENTR-OsMYB55	Km ^R , pENTR/D-TOPO containing <i>OsMYB55</i> CDS	This study
pUbi-GUS	Amp ^R , pUbi_RfA containing 1.8 kb <i>GUS</i> fragment from pENTR-GUS	15
pUbi-OsMYC2	Amp ^R , pUbi-RfA-Tnos containing <i>OsMYC2</i> CDS	This study
pUbi-OsMYL1	Amp ^R , pUbi-RfA-Tnos containing <i>OsMYL1</i> CDS	This study
pUbi-OsMYL2	Amp ^R , pUbi-RfA-Tnos containing <i>OsMYL2</i> CDS	This study
pUbi-JAdMYB1	Amp ^R , pUbi-RfA-Tnos containing <i>JAdMYB1</i> CDS	This study
pUbi-JAdMYB2	Amp ^R , pUbi-RfA-Tnos containing <i>JAdMYB2</i> CDS	This study
pUbi-JAdMYB3	Amp ^R , pUbi-RfA-Tnos containing <i>JAdMYB3</i> CDS	This study
pUbi-JAdMYB4	Amp ^R , pUbi-RfA-Tnos containing <i>JAdMYB4</i> CDS	This study
pUbi-JAdMYB5	Amp ^R , pUbi-RfA-Tnos containing <i>JAdMYB5</i> CDS	This study
pUbi-OsJAmyb	Amp ^R , pUbi-RfA-Tnos containing <i>OsJAmyb</i> CDS	This study
pUbi-OsMYB55	Amp ^R , pUbi-RfA-Tnos containing <i>OsMYB55</i> CDS	This study
pENTR-OsMYC2 RNAi	Km ^R , pENTR/D-TOPO containing 748-bp trigger region of <i>OsMYC2</i>	This study
pANDA	Hm ^R , Km ^R , Cm ^R , <i>ccdB</i> , maize <i>ubiquitin</i> promoter-Gateway (reverse)- <i>gus</i> linker-Gateway- <i>NOS</i> terminator,	32
pANDA-OsMYC2	Hm ^R , Km ^R , pANDA containing 748-bp trigger region of <i>OsMYC2</i>	This study

pnYGW	Amp ^R , Cm ^R , <i>ccdB</i> , CaMV 35S promoter- nEYFP-Gateway- <i>NOS</i> terminator	34
pcYGW	Amp ^R , Cm ^R , <i>ccdB</i> , CaMV 35S promoter- cEYFP-Gateway- <i>NOS</i> terminator	34
pnYOsMYC2	Amp ^R , pnYGW containing <i>OsMYC2</i> CDS	This study
pcYOsMYC2	Amp ^R , pcYGW containing <i>OsMYC2</i> CDS	This study
pcYOsMYL1	Amp ^R , pcYGW containing <i>OsMYL1</i> CDS	This study
pcYOsMYL2	Amp ^R , pcYGW containing <i>OsMYL2</i> CDS	This study
pTH121R	Amp ^R , CaMV 35S promoter- <i>DsRed1</i> - <i>NOS</i> terminator	17
pENTR-optOsMYC2	Km ^R , pENTR/D-TOPO containing optimized <i>OsMYC2</i> CDS	This study
pEU-E01-6His-Bls-GW-STOP	Amp ^R , Cm ^R , <i>ccdB</i> , SP6 promoter-E01 translational enhancer-His tag-biotin ligation site-Gateway	This study
pEU-E01-FLAG-GW-STOP	Amp ^R , Cm ^R , <i>ccdB</i> , SP6 promoter-E01 translational enhancer-FLAG tag-Gateway	35
pEU-HisBls-optOsMYC2	Amp ^R , pEU-E01-6His-Bls-GW containing optimized <i>OsMYC2</i> CDS	This study
pEU-FLAG-optOsMYC2	Amp ^R , pEU-E01-FLAG-GW containing optimized <i>OsMYC2</i> CDS	This study
pEU-FLAG-OsMYL1	Amp ^R , pEU-E01-FLAG-GW containing <i>OsMYL1</i> CDS	This study
pEU-FLAG-OsMYL2	Amp ^R , pEU-E01-FLAG-GW containing <i>OsMYL2</i> CDS	This study
430T1.2	Amp ^R , CaMV 35S promoter-GAL4 DNA binding domain- <i>NOS</i> terminator	36
p35S-GAL-OsMYC2	Amp ^R , 430T1.2 containing <i>OsMYC2</i> CDS	This study
p35S-GAL-OsMYL1	Amp ^R , 430T1.2 containing <i>OsMYL1</i> CDS	This study
p35S-GAL-OsMYL2	Amp ^R , 430T1.2 containing <i>OsMYL2</i> CDS	This study
GAL4-TATA-LUC-NOS	Amp ^R , GAL4 <i>cis</i> -firefly <i>LUC</i> - <i>NOS</i> terminator	28

Supplementary Table S4 Primers used for cloning.

Primer	Sequence
RLUC Fw	5'-CACCATGACTTCGAAAAGTTTATGATCCA-3'
RLUC Rv	5'-TTATTGTTCATTTTGAGAACTCGCT-3'
OsNOMT ₁₀₀₀ Fw	5'-AAAGGTACCATAAATCTCCCATATATGTAGACAGTTTC-3'
OsNOMT ₁₀₀₀ Rv	5'-GGGGAGCTCGCTACTATACGGGGACACTG-3'
OsMYC2 Fw	5'-CACCATGAACCTTTGGACGGACGACAACGCGTC-3'
OsMYC2 Rv	5'-TTACCGGGCGGCGGTGCC-3'
OsMYL1 Fw	5'-CACCATGTCGTGGTCCGAGACG -3'
OsMYL1 Rv	5'-TCACGGGGAGGGAGTGGTG -3'
OsMYL2 Fw	5'-CACCATGGTGATGAAGATGGAGGC -3'
OsMYL2 Rv	5'-CTACCCTGAGTTCATCACGC -3'
JAdMYB1 Fw	5'-CACCATGGGACGGCACGCGTGCTC -3'
JAdMYB1 Rv	5'-TCATCGGAAGTACTCGAAGTTGAAGTCGCCGAC -3'
JAdMYB2 Fw	5'-CACCATGGCAGCATCGCAGAGGAG -3'
JAdMYB2 Rv	5'-TCATCGATTCCATATATAATTCGAATCAAATCGACATCG -3'
JAdMYB3 Fw	5'-CACCATGGCCAGGAAATGCTCCAGCTG -3'
JAdMYB3 Rv	5'-TCAAGTGACCCTGATTGTCCCCAAAAAAGG -3'
JAdMYB4 Fw	5'-CACCATGGGGAGGTGCGCCGTGCTG -3'
JAdMYB4 Rv	5'-TCATATGTACTGGCCTTGTTC AACGGCCTG -3'
JAdMYB5 Fw	5'-CACCATGGGGAGGTGCGCCGTGCTG -3'
JAdMYB5 Rv	5'-TCATTTTCATGGGGAGGCTTCTGAAGTCGAGC -3'
OsJAmyb Fw	5'-CACCATGGAGATGGTGCTGCAGAGGAC -3'
OsJAmyb Rv	5'-TTATTGCATCTTCCATATGTCCTAGACTCCATAGAG -3'
OsMYB55 Fw	5'-CACCATGGGGCGCGCCGTGCTG -3'
OsMYB55 Rv	5'-CTATGTCAGGGTGTTCAGAGACCCTGTGGCTTGG -3'
OsMYC2 RNAi Fw	5'-CACCCCTCTACATCAACGAGCTCC-3'
OsMYC2 RNAi Rv	5'-ACAAGGACCAACATATCGCC-3'
pEU-bls-InFusion Fw	5'-AACGACATCTTCGAGGCCAGAAAGATCGAGTGGCACGAAACAAGTTTGTACAAAAAAGC-3'
pEU-bls-InFusion Rv	5'-CTCGAAGATGTCGTTCAAGCCATGATGGTGATGGTGATGCATGATATCTTGGTGATGTAG-3'
optOsMYC2 Fw 1-	5'-CACCATGAACCTCTGGACCGATGATAACG-3'
optOsMYC2 Fw 671-	5'-GTGTTCTCGAGCTTGGATCTACCGATGTGATCTTCCAGAC-3'
optOsMYC2 Rv	5'-TCATCTAGCAGCAGTACCAGGTTC-3'
OsMYC2 GAL4 Fw	5'-GGAGGCCGAATTC CCCGGG GATGAACCTTTGGACGGACGACAACGCGTC-3'
OsMYC2 GAL4 Rv	5'-GGTCGACGGAT CCCGGG TACCGGGCGGCGGTGCC-3'
OsMYL1 GAL4 Fw	5'-GGAGGCCGAATTC CCCGGG GATGTCGTGGTCCGAGACGGACG-3'
OsMYL1 GAL4 Rv	5'-GGTCGACGGAT CCCGGG TACCGGGAGGGAGTGGTGAC-3'

OsMYL2 GAL4 Fw 5'-GGAGGCCGAATTCCCCGGGGATGGTGATGAAGATGGAGGCTGATG-3'

OsMYL2 GAL4 Rv 5'-GGTCGACGGATCCCCGGGCTACCCTGAGTTCATCACGCGAG-3'

Restriction sites are underlined. Bls-tag epitope sequence is shaded. His tag sequence is shown in bold.

Supplementary Table S5 **Primers used for expression analyses.**

Primer	Sequence	Target gene
OsUBQ RT Fw	5'-TCCGAGAGATGGGTTTCATC -3'	<i>OsUBQ</i>
OsUBQ RT Fw	5'-GCCAAGATTGCCAAGAAGAC-3'	<i>OsUBQ</i>
OsMYC2 Fw	5'-AGCTCAACCAGCGCTTCTAC-3'	<i>OsMYC2</i>
OsMYC2 Rv	5'-GTCTCCTTGTCGGTCTCCAG-3'	<i>OsMYC2</i>
OsNOMT Fw	5'-AAGGTGTTTCATGGAGAAGTGGTA-3'	<i>OsNOMT</i>
OsNOMT Rv	5'-CTGGTTGAAGAGCGTGTGGA-3'	<i>OsNOMT</i>

Supplementary Table S6 **Vectors referred to in the *Supplementary methods* section.**

Vector	Relevant characteristics	Reference
pUbi-RLUC	Amp ^R , pUbi-RfA-Tnos containing <i>renilla luciferase</i> CDS	This study
pZErO2	Km ^R , <i>ccdB</i> , cloning vector	Invitrogen
pZErO2-OsNOMT ₂₅₀	Km ^R pZErO2 containing 5' 250-bp upstream region of <i>OsNOMT</i>	This study
pZErO2-OsNOMT ₁₅₀	Km ^R pZErO2 containing 5' 150-bp upstream region of <i>OsNOMT</i>	This study
pZErO2-OsNOMT ₅₀	Km ^R pZErO2 containing 5' 50-bp upstream region of <i>OsNOMT</i>	This study
pZErO2-OsNOMT ₂₅₀₋₂₆	Km ^R pZErO2 containing 5' 250–26-bp upstream region of <i>OsNOMT</i>	This study
pGL4-Tnos	Amp ^R , Firefly <i>LUC-NOS</i> terminator	This study
pGL4-OsNOMT ₂₅₀	Amp ^R , 5' 250-bp upstream region of <i>OsNOMT</i> - Firefly <i>LUC-NOS</i> terminator	This study
pGL4-OsNOMT ₁₅₀	Amp ^R , 5' 150-bp upstream region of <i>OsNOMT</i> - Firefly <i>LUC-NOS</i> terminator	This study
pGL4-OsNOMT ₅₀	Amp ^R , 5' 50-bp upstream region of <i>OsNOMT</i> - Firefly <i>LUC-NOS</i> terminator	This study
pGL4-OsNOMT ₂₅₀₋₂₆	Amp ^R , 5' 250–26-bp upstream region of <i>OsNOMT</i> - Firefly <i>LUC-NOS</i> terminator	This study
pUbi-GUS	Amp ^R , pUbi_RfA containing 1.8 kb <i>GUS</i> fragment from pENTR-GUS	15
pUbi-OsMYC2	Amp ^R , pUbi-RfA-Tnos containing <i>OsMYC2</i> CDS	This study

Supplementary Table S7 **Primers used for cloning and referred to in the *Supplementary methods* section.**

Primer	Sequence
OsNOMT ₂₅₀ Fw	5'-AAACTC <u>GAG</u> TAGGAAGTTTAATGGACTTTTAGTAGATAATAG-3'
OsNOMT ₂₅₀ Rv	5'-GGGAGATC <u>TGCT</u> ACTATACGGGGACAC-3'
OsNOMT ₁₅₀ Fw	5'-AAACTC <u>GAG</u> AACGTTGGAAAAAACCAACTACAC-3'
OsNOMT ₁₅₀ Rv	5'-GGGAGATC <u>TGCT</u> ACTATACGGGGACAC-3'
OsNOMT ₅₀ Fw	5'-AAACTC <u>GAG</u> AGCTAGGTATCCTAGCTATATAAAC-3'
OsNOMT ₅₀ Rv	5'-GGGAGATC <u>TGCT</u> ACTATACGGGGACAC-3'
OsNOMT ₂₅₀₋₂₆ Fw	5'-TAGGAAGTTTAATGGACTTTTAGTAGATAATAGATAAATAG-3'
OsNOMT ₂₅₀₋₂₆ Rv	5'-GTTTATATAGCTAGGATACCTAGCTTATTGG-3'

Restriction sites are underlined.