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Supplemental information

Inheritance of acquired adaptive cold tolerance in rice through DNA methylation

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Table S1. DNA methylation status and cold-induced expression change of *ACT1* as well as cold tolerance abilities in 31 rice accessions, related to Figure 1 and 4.

Sample ID	Name	¹ Average mCG percentage	Average mCHG percentage	² <i>ACT1</i> DNA methylation status	<i>ACT1</i> expression change (Cold/normal)	Average seed setting ratio (Cold/normal)
CT1	Zhonghua 11	23%	4.3%	Low	1.216036	90.60381
CT2	TEJ 3	29%	4.3%	Low	1.060948	86.54913
CT3	Hejiang 1	37%	12%	Low	1.0311	85.61656
CT4	Dongnong 423	29%	4.3%	Low	1.151376	85.50336
CT5	Songjing 8	14%	2%	Low	1.106109	84.00111
CT6	Longdao 7	39%	10%	Low	1.251732	83.81573
CT7	Fushiguang	27%	8.3%	Low	1.376667	83.54833
CT8	Jijing 81	34%	9.3%	Low	1.062157	82.94226
CT9	Qingniantehao	15%	2.3%	Low	0.671994	81.96769
CT10	Wunong 71	21%	3.3%	Low	1.240079	80.88437
CT11	Jijing 83	33%	11.3%	Low	1.313107	80.25284
CT12	Yanjing 19	39%	11%	Low	0.655268	77.51614
CT13	Longdao 3	17%	2.6%	Low	0.967181	75.36484
CT14	Longjing 14	33%	4.3%	Low	0.864421	73.28343
CT15	Songjing 3	49%	12.3%	High	0.726135	70.4618
CT16	Songjing 10	40%	8%	Low	0.955048	68.70811
CT17	Baidadu	23%	6.3%	Low	0.895706	68.68423
CT18	Longjing 16	26%	5%	Low	0.915449	66.55606
CT19	Longdao 5	24%	6.7%	Low	0.936131	65.57968
CT20	Dalinian	23%	5.3%	Low	0.663212	64.88062
CT21	Hongmang	45%	16%	High	0.757594	64.19142
CT22	Bairen	18%	3.6%	Low	0.923623	63.5905
CS1	Shangyu 397	63%	19.7%	High	0.67509	39.74323
CS2	Yanjing 16	74%	15.3%	High	0.53194	39.20626
CS3	Dashengchan	63%	16.7%	High	0.551471	38.38215
CS4	Kendao 12	73%	22%	High	0.437676	37.39969
CS5	Piaohonggenniandao	73%	13.7%	High	0.476096	28.6539
CS6	Sanjiang 1	56%	19.7%	High	0.470284	23.99671
CS7	Kendao 8	78%	30.7%	High	0.315738	12.60636
CS8	Zhongmunong 8	69%	29.7%	High	0.287319	8.893762
CS9	Longjing 11	72%	22.7%	High	0.260797	6.253577

¹ The mC percentage at the –1,549 to –1,513 bp upstream of *ACT1* revealed by bisulfite sequencing.

² Based on the average mC percentage at the –1,549 to –15,13 bp upstream of *ACT1* in KD8-4N (mCG, 68.3%; mCHG, 25%) and KD8-4C (mCG, 24.6%; mCHG, 1.75%) (Fig. 2D), the DNA methylation level of these rice varieties was defined as either low [average mCG percentage < (KD8-4C+KD8-4N) /2, 46.4%; mCHG, 13.4%] or high [mCG percentage >46.4; mCHG percentage >13.4%].

Table S2. Cold tolerance abilities and DNA methylation status in *ACT1* among the progenies from F₂ population generated by reciprocal crosses between KD8-4N and KD8-4C, related to Figure 2.

¹ Sample ID	Average seed setting ratio upon cold	² Average mCG percentage	Average mCHG percentage	Cold tolerance
KD8-4N	0.2073	68.3%	25%	CS
KD8-4C	0.7031	24.6%	1.7%	CT
HT176-67	0.906481481	49.3%	12.7%	CT
HT176-54	0.878733572	27%	5.6%	CT
HT177-251	0.824499364	22.3%	4%	CT
HT176-256	0.8177	15.7%	6.7%	CT
HT176-218	0.817330097	37%	9.3%	CT
HT177-183	0.806693307	3.3%	1.7%	CT
HT176-14	0.799024486	3.3%	1.3%	CT
HT177-106	0.796434996	48.3%	5.3%	CT
HT176-255	0.783419857	31.6%	5.6%	CT
HT176-172	0.753644094	27.6%	7.7%	CT
HT176-114	0.7527	46%	8.7%	CT
HT176-135	0.746478873	38.3%	9%	CT
HT176-52	0.745762712	29%	6.3%	CT
HT176-173	0.745563373	19.7%	3%	CT
HT177-213	0.745283019	5%	1.7%	CT
HT176-251	0.7414	20.7%	2.3%	CT
HT176-61	0.726943347	25%	6%	CT
HT176-186	0.71875	22.3%	5.7%	CT
HT176-243	0.712652965	31.3%	9.3%	CT
HT177-256	0.711393869	44.3%	6.3%	CT
HT176-176	0.191109669	59.3%	13.3%	CS
HT177-137	0.1901	65%	13%	CS
HT176-185	0.179406037	52.3%	13.3%	CS
HT176-151	0.174236053	60.7%	15.7%	CS
HT177-35	0.167679992	66.3%	18.3%	CS
HT177-64	0.165714286	56.7%	9.3%	CS
HT176-152	0.148090005	56.3%	21.7%	CS
HT176-22	0.146341463	59.7%	13.3%	CS
HT176-237	0.140625	55%	11.3%	CS

HT177-172	0.12194211	33%	8.7%	CS
HT176-112	0.111423221	58%	11%	CS
HT177-33	0.106902357	66.7%	10%	CS
HT177-97	0.07594714	66%	13.7%	CS
HT176-66	0.073780488	61%	17.3%	CS
HT176-84	0.071044547	69.3%	16.3%	CS
HT177-77	0.058320479	26.7%	8%	CS
HT177-171	0.045707915	66.3%	11.3%	CS
HT176-71	0.025316456	61.3%	11.6%	CS

¹ HT176, the progenies form the cross of ♀ KD8-4N × ♂ KD8-4C; HT177, the progenies form the cross of ♀ KD8-4C × ♂ KD8-4N.

² The mC percentage at the −1,549 to −1,513 bp upstream of *ACT1* revealed by bisulfite sequencing.

Table S4. Primers used in this study, related to STAR METHODS.

Primer ID	Primers sequence(5'-3')	Purpose
OsU3-F	CCCCTTTCGCCAGGGGTACC gtaattcatccagggtccaag	Generation of <i>ACT1</i> knock-out construct
OsU3-R	TACGAATTCGAGCTCGGTACC gctgtgccgtacgacggtacg	Generation of <i>ACT1</i> knock-out construct
ACT1-F	aggcgtcgtcactggcggt GTTTTAGAGCTAGAAATAGCAAGTTA	Generation of <i>ACT1</i> knock-out construct
ACT1-R	agccgccagtgcagcgcct gccacggatcatctgcacaac	Generation of <i>ACT1</i> knock-out construct
CP6248	ggcaCGATCCACCAGCAGCGACGG	OsU3T1F for <i>Dof1</i> to generate pYL-Cas9-H
CP6249	aaacCCGTCGCTGCTGGTGGATCG	OsU3T1R for <i>Dof1</i> to generate pYL-Cas9-H
CP6250	gccgTGGATCGCCGGTACAAGCA	OsU6aT2F for <i>Dof1</i> to generate pYL-Cas9-H
CP6251	aaacTGCTTGTACCGGCGATCCA	OsU6aT2R for <i>Dof1</i> to generate pYL-Cas9-H
CP2558	AAGCCCACCAACCTCAAAAAC	Genotyping of <i>ACT1</i> knock-out lines
CP2559	TGAGGAGCTAGATCAGAACAGA	Genotyping of <i>ACT1</i> knock-out lines
XP0971	AAAAACTCCCACCCTGTGCTA	Genotyping of <i>Dof1</i> knock-out lines
XP0972	CGGCATCCCTTGCAAGTAG	Genotyping of <i>Dof1</i> knock-out lines
CF6964	gacacggggtgttgggtt tatgtacagcattacgtagg	Generation of <i>ACT1</i> epigenome editing construct
CF6965	catctatgttactgttgggt gatgggtcctactgtttag	Generation of <i>ACT1</i> epigenome editing construct
CF8414	gtcactgtcaaaaagttcag GTTTTAGAGCTAGAAATAGCAAGTTA	Generation of <i>ACT1</i> epigenome editing construct
CF8415	ctgaacttttgacagtgc AACCTGAGCCTCAGCGCAGC	Generation of <i>ACT1</i> epigenome editing construct
CP5290	GGGACACGTCGGACCTTAT	<i>AciI</i> Chop-PCR amplification primer
CP5291	TGACACTAGATGAGTAGTAGGAGT	<i>AciI</i> Chop-PCR amplification primer
CP5356	YGTTAAAGAATTGGTTTTGAT	<i>ACT1</i> bisulfite PCR amplification primer
CP5357	AACATAACACCTTTTAAACCATA	<i>ACT1</i> bisulfite PCR amplification primer
HX9046	GCCCAAGAAGAAGATCAAGAAC	RT-qPCR primer for <i>UBQ</i>
HX9047	AGATAACAACGGAAGCATAAAAGTC	RT-qPCR primer for <i>UBQ</i>
CF8657	gtggcgccggtgtctct	RT-qPCR primer for <i>ACT1</i>
CF8658	gtcggggactgggagatcg	RT-qPCR primer for <i>ACT1</i>
CF8653	GACCGTAAGGGAGAATGC	RT-qPCR primer for <i>CMT3</i>
CF8654	CTATTGCTCAACAACCTCGC	RT-qPCR primer for <i>CMT3</i>
CF8655	GATTGGCCAGAACCAATG	RT-qPCR primer for <i>MET1b</i>
CF8656	TGATCATTACGCGCATCG	RT-qPCR primer for <i>MET1b</i>
CF9200	actctagaggatcaattc atggcgcgcctccacctcg	Generation of <i>ACT1</i> overexpression construct
CF9201	atccttgaatctcccat gtagaacgccgcgacggctg	Generation of <i>ACT1</i> overexpression construct
XP3067	ttctgcagcccggtgatcc ATGCTGCCGTACGCGC	Generation of <i>ZmUBI;Dof1-FLAG</i> construct
XP3068	gatcggggaaattcggtatcc tcagcattgtcgtcatcgtct	Generation of <i>ZmUBI;Dof1-FLAG</i> construct
CF8768	ccgCTCGAG GTACCAAGCACGACTTGGA	Amplified the <i>OsCMT3</i> RNAi region
CF8769	cgGGATCC GTGATCAAAAAGCTTGCCTAG	Amplified the <i>OsCMT3</i> RNAi region
CF8770	ccgCTCGAG ATTTGCCTGATGGTAAATAC	Amplified the <i>OsMET1b</i> RNAi region
CF8771	cgGGATCC CAATCCAGCCTACCATAAAG	Amplified the <i>OsMET1b</i> RNAi region
CP7868	ggagtgtacgtgtgtgc agtaagatgYYgtgagaaaa	<i>ACT1</i> bisulfite PCR amplification primer with Hi-TOM adaptor

CP7869	gagttggatgctggatgg AACATAACACCTTTTAAACCATA	<i>ACT1</i> bisulfite PCR amplification primer with Hi-TOM adaptor
XP0020	tgggcggtggeggtatccggg GAGACAATTCGTTTGCCCAAACC	Generation of <i>ACT1</i> DNA methylation editing construct
XP0021	gatcggggaaatcggtatcc tcaATGTTTATGTCTGGACATTATGG	Generation of <i>ACT1</i> DNA methylation editing construct
CP7352	cgtctagaactagtggatcc ctgccgtacgcgccgcgg	Generation of GAL4 DBD- <i>Dof1</i> construc
CP7353	ttgcggagtacccgggtacc ttatggcatgtaaagagcctcc	Generation of GAL4 DBD- <i>Dof1</i> construc
XP4489	ACGGGATTCTGAACCTTGAC	ChIP-qPCR primer for R1 region
XP4490	CCGCAAATCACACGTCATGC	ChIP-qPCR primer for R1 region
XP4483	CATGACGTGTGATTTGCGGG	ChIP-qPCR primer for R2 region
XP4484	TCTAGAGTAGTCGCCGCTGA	ChIP-qPCR primer for R2 region
XP4491	CGGACGCGCATTATCACAAA	ChIP-qPCR primer for R3 region
XP4492	TCACCATGGGCAAGTGAAGT	ChIP-qPCR primer for R3 region
XP4487	GCACCGTTTCGTTTGTTC	ChIP-qPCR primer for R4 region
XP4488	TTGGACGGTGATTACGCCAT	ChIP-qPCR primer for R4 region