

Sustained low abscisic acid levels increase seedling vigor under cold stress in rice (*Oryza sativa* L.)

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Supplementary Data

Supplementary Table SI. List of primers used in this study.

Supplementary Table SII. Genes with differential expression between transgenic rice (E0082::ABA8ox1 27-3) and wild-type seedlings under cold stress (15°C).

Supplementary Figure S1. Distribution of *cis*-acting elements in the promoter region of *OsABA8ox1*, *OsABA8ox2*, and *OsABA8ox3*.

Supplementary Figure S2. Expression of *OsABA8oxs*, *OsNCEDs*, *OsABA2*, and *OsAAOs* under long-term cold stress.

Supplementary Figure S3. Growth and *OsABA8ox1* gene expression of E0082::ABA8ox1 11-22 and E0082::ABA8ox1 49-38.

Supplementary Figure S4. Sterility rate of E0082::ABA8ox1 25-20 and E0082::ABA8ox1 27-3.

Supplementary Table SI. List of primers used in this study

Experiment	Gene	Locus/Accession No.	Forward primer	Reverse primer	TaqMan probes
Transgenic Arabidopsis	<i>OsABA8ox1</i>	Os02g0703600	caccatgccctgcgtgatg	ctcctgctcggtgttcttg	
	<i>OsABA8ox2</i>	Os08g0472800	caccatggctttcttgccttcttctgt	agcacctgctgctgtgatg	
	<i>OsABA8ox3</i>	CI523426	caccatggcagcctccttcgtc	aaccattgctacgccattgtc	
Transgenic rice	<i>OsABA8ox1</i>	Os02g0703600	ggttccgcgtggatccatgggtgct tttcttctgttcg	gtcgaccegggaattctcactcctgc tcggtgttct	
QRT-PCR	<i>OsABA8ox1</i>	Os02g0703600	tcaacacctccaagagatgaa	atctctctccccgaag	#56
	<i>OsABA8ox2</i>	Os08g0472800	ggcgagcataatctcctca	ccctttggaatcaggaaacc	#34
	<i>OsABA8ox3</i>	CI523426	catggcctaaccacaa	gggataaggaacccttgtactc	#159
	<i>Rab16A</i>	Os11g0454300	gaggaggagcacaagacc	attccatcatcctcagacgag	#78
	<i>25S rRNA</i>	AK119809	ctcttttccccatgcac	agcaatctgtctgctcatcaag	#82
	<i>OsNCED1</i>	CI049010	tcgcatcaccgagaacta	tctctggagcttgaacacc	#131
	<i>OsNCED2</i>	Os12g0617400	aagaagaaggatgggctgaac	tgatgaacccttctcgaac	#11
	<i>OsNCED3</i>	Os07g0154100	ccctaccagtcctgtgc	gcccatggaagtcttacct	#152
	<i>OsABA2</i>	Os03g0810800	tggagctgcaattagtgagg	ccacagtcgtacacgaggtc	#119
<i>OsAAOs</i>	Os03g0790900/ Os10g0138100	gctctctccatgccgtatct	ccacaccttcaggtttgct	#157	

Supplementary Table SII. Genes with differential expression between transgenic rice (E0082::ABA8ox1 27-3) and wild-type seedlings under cold stress (15°C)

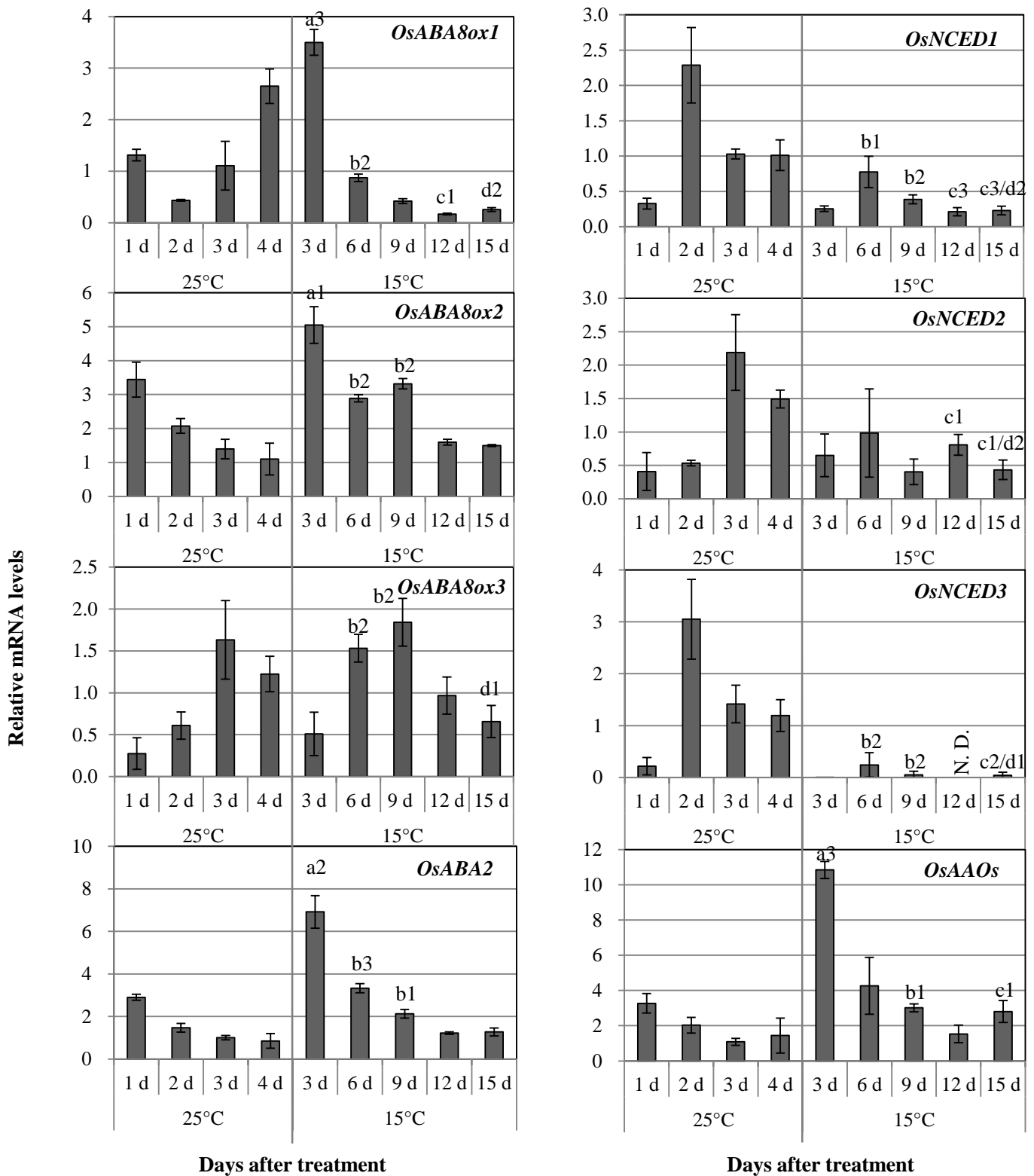
ProbeName	Toyohikari				27-3				Rice X pro data		Description	PrimaryAccession	Reference
	25°C2d	25°C3d	15°C9d	15°C15d	25°C2d	25°C3d	15°C9d	15°C15d	ABA 6h	ABA 12h			
Transcription factor													
RICEGENE13573	987.0	706.0	1595.6	1489.0	287.4	355.8	1585.5	622.6	7.2		OSBZ8. [Os01t0658900-01]	Os01g0658900_AK121925	1
RICEGENE30459	361.2	101.7	15.3	16.2	21.2	24.2	17.7	30.0	5.9		Zinc finger, C2H2-type domain containing protein. [Os03t0437200-01]	Os03g0437200_AY295345	
RICEGENE08765	472.9	78.3	6.6	13.8	10.7	12.4	14.6	14.6	20.2		Zinc finger, C2H2-type domain containing protein. [Os03t0437200-01]	Os03g0437200_AK059839	
RICEGENE17191	2911.7	1108.2	3083.6	3557.4	575.3	377.2	2601.1	2774.9	9.9	9.2	Zinc finger, CCCH-type domain containing protein. [Os05t0195200-01]	Os05g0195200_AK106392	
RICEGENE23696	964.3	661.3	1345.5	1145.1	354.6	342.3	1090.4	665.1	7.4	6.5	Similar to Myb-related transcription factor LBM1. [Os07t0558100-00]	Os07g0558100_Y11415	
RICEGENE36837	124.3	129.3	284.0	381.4	27.0	54.1	347.6	413.0	2.3	2.1	Similar to Heat shock transcription factor 29 (Fragment). [Os01t0733200-01]	Os01g0733200_AK066316	
RICEGENE10967	138.7	362.4	1057.8	745.0	289.4	327.8	464.4	303.7	2.7	3.7	Similar to Heat stress transcription factor B-2b. [Os08t0546800-01]	Os08g0546800_AK101700	
RICEGENE04745	3739.5	3455.3	3363.1	3607.7	1576.9	2363.3	1941.8	1942.8	4.7	3.2	Similar to Abscisic acid responsive elements-binding factor (ABA-responsive element binding protein 2) (AREB2). [Os02t0766700-01]	Os02g0766700_AK072062	
RICEGENE27103	21.7	41.3	35.4	34.5	17.3	17.1	21.2	852.1		2.9	Similar to Abscisic acid responsive elements-binding factor. [Os06t0724000-01]	Os06g0724000_AK108991	
PP2C													
RICEGENE06434	484.4	562.8	321.0	382.6	215.5	224.5	326.0	237.3	36.7	50.4	Protein phosphatase 2C family protein. [Os06t0698300-01]	Os06g0698300_AK071637	
RICEGENE32168	690.3	791.7	565.9	409.5	262.9	320.9	336.0	262.1	3.9	7.0	Protein phosphatase 2C family protein. [Os01t0656200-01]	Os01g0656200_AK068272	2
RICEGENE08607	5623.0	4478.9	7694.8	6999.8	2230.8	1966.7	4791.0	4121.9	6.5	6.7	Similar to Protein phosphatase 2C. [Os01t0583100-01]	Os01g0583100_C1535252	2
RICEGENE26038	1017.1	984.2	1017.8	1546.3	240.7	227.6	510.7	339.4	12.2	8.5	Similar to protein phosphatase 2C. [Os01t0846300-02]	Os01g0846300_AK065949	2
RICEGENE16735	823.3	736.4	527.6	1161.8	54.8	140.0	833.5	182.0	13.8	14.0	Similar to Protein phosphatase type 2C. [Os03t0268600-01]	Os03g0268600_AK069274	2
RICEGENE03743	5560.3	2837.3	2816.3	2344.7	353.7	306.9	1776.8	277.3	75.0	60.9	Similar to Protein phosphatase 2C (PP2C) (EC 3.1.3.16). [Os09t0325700-01]	Os09g0325700_AK063334	2
Water stress													
RICEGENE25055	2314.5	6713.1	4723.5	7364.1	70.5	182.0	459.7	691.6	85.5	76.7	Similar to WCOR719. [Os03t0820500-01]	Os03g0820500_C1417173	
RICEGENE15477	64.2	156.9	59.9	24.7	11.3	16.6	20.2	16.9	9.3		WSI76 protein induced by water stress. [Os07t0687900-01]	Os07g0687900_AK107065	
RICEGENE37809	54.4	82.3	60.9	23.1	10.3	19.5	15.4	14.0	8.6		WSI76 protein induced by water stress. [Os07t0687900-01]	Os07g0687900_D26537	
RICEGENE06777	512.3	1622.6	334.9	138.6	89.7	110.3	111.2	53.6	82.2	174.7	Dehydrin RAB 16B. [Os11t0454200-01]	Os11g0454200_AK063517	3
RICEGENE04011	59.4	522.9	9.0	17.6	11.0	12.6	14.6	68.5			Dehydrin RAB 16C. [Os11t0454000-01]	Os11g0454000_AK071366	3
RICEGENE04307	95.9	1279.3	30.4	84.8	10.9	21.6	28.4	12.3			Dehydrin RAB 16D. [Os11t0453900-01]	Os11g0453900_AK109096	3
RICEGENE05739	427.3	2463.9	171.0	8.9	11.6	13.6	15.5	15.6	499.1	560.2	Similar to Dehydrin DHN1 (M3) (RAB-17 protein). [Os11t0451700-00]	Os11g0451700_C1050356	
RICEGENE29579	7.1	428.7	58.4	1241.9	29.0	366.6	14.2	1871.4	2.3		ABA/WDS induced protein family protein. [Os01t0963600-01]	Os01g0963600_AK104613	
RICEGENE13810	1499.1	14149.5	3503.6	345.4	52.2	62.1	232.6	230.2	8.0	9.5	Similar to Water-stress inducible protein RAB21. [Os11t0454300-01]	Os11g0454300_AK121952	
RICEGENE02055	7.6	16.1	6.9	8.6	10.4	12.1	13.6	146.7	6.5	8.6	Rep: Dehydrin Rab25 - Oryza sativa subsp. japonica (Rice), complete [TC524743]	Os01g0702500_AY333185	
RICEGENE27617	22.9	126.6	45.0	21.5	45.6	49.8	33.7	25.2	3.9	4.0	Similar to Dehydrin Rab25. [Os01t0702500-01]	Os01g0702500_AK063691	
RICEGENE22964	479.0	234.0	225.2	212.4	142.0	121.3	214.2	234.8	2.3		Similar to Early response to dehydration 15-like protein. [Os03t0353400-02]	Os03g0353400_AK068753	

Heat shock													
RICEGENE26600	549.8	963.5	1103.7	1446.9	204.0	246.7	758.4	332.6	11.2	9.6	16.9 kDa class I heat shock protein 1. [Os01t0136100-01]	Os01g0136100_X60820	
RICEGENE33820	770.5	1317.9	3510.8	4105.2	306.9	648.7	1061.7	1332.9	7.9	12.4	Similar to 17.5 kDa class II heat shock protein. [Os02t0782500-02]	Os02g0782500_AK063602	
RICEGENE07742	20821.0	30410.9	39464.8	38549.0	21157.5	30415.1	28387.5	15006.9	2.2	4.0	Heat shock protein Hsp70 family protein. [Os01t0840100-01]	Os01g0840100_C1273581	4
RICEGENE22604	11.6	9.7	13.3	8.3	46.2	11.6	30.1	13.8	2.4	5.4	Heat shock protein Hsp70 family protein. [Os03t0276800-01]	Os03g0276800_AK106371	
RICEGENE37205	142.5	501.2	103.1	112.3	96.5	293.4	46.5	52.1			Heat shock protein 70. [Os03t0277300-01]	Os03g0277300_C1066180	4
RICEGENE20721	186.9	371.1	392.3	308.9	156.5	219.9	201.5	111.6			Rep: HSP70 - Triticum aestivum (Wheat), complete [TC515901]	Os05g0460000_C1368692	4
RICEGENE17769	27.9	52.4	187.8	178.7	49.7	26.1	1036.6	76.6	11.4	35.1	Similar to Heat shock protein 80. [Os04t0107900-03]	Os04g0107900_AK063751	
RICEGENE19320	18.1	37.5	144.5	153.9	11.1	15.8	71.6	718.8	13.8	34.3	Similar to Heat shock protein 82. [Os04t0107900-01]	Os04g0107900_AK063698	
RICEGENE21268	7.8	8.9	20.0	26.2	9.4	42.5	16.0	14.3		5.2	Similar to Heat shock protein 82. [Os04t0107900-04]	Os04g0107900_AK063629	
RICEGENE11851	127.3	213.0	976.6	1326.4	77.3	175.4	372.1	446.4	6.2	9.7	Heat shock protein 101. [Os05t0519700-02]	Os05g0519700_AF332981	
RICEGENE33566	329.7	563.4	2977.9	4493.9	216.5	226.1	962.2	1054.7	9.0	10.4	Similar to Heat shock protein 101. [Os05t0519700-03]	Os05g0519700_AK121414	
RICEGENE01989	70.4	144.0	676.2	871.7	46.5	47.9	232.4	208.1		10.4	Heat shock protein 101. [Os05t0519700-01]	Os05g0519700_AK105433	
RICEGENE24082	45.2	138.0	64.4	8.2	9.8	11.4	13.4	13.8	69.2	95.7	Similar to Low-temperature induced protein It101.2. [Os05t0122700-01]	Os05g0122700_C1441278	
RICEGENE34540	349.9	843.3	338.5	269.5	102.4	320.1	172.5	214.0	59.9	84.1	Similar to Low-temperature induced protein It101.2. [Os03t0286900-01]	Os03g0286900_AK070872	
RICEGENE26571	487.4	832.8	2075.0	2515.4	182.6	399.0	643.0	818.4	11.0	18.8	Similar to Small heat stress protein class CIII. [Os02t0782500-01]	Os02g0782500_AK119261	
RICEGENE30579	44.2	258.5	134.4	16.6	8.9	10.5	16.8	14.0	126.1	234.1	Similar to Cor14b protein precursor. [Os04t0610600-01]	Os04g0610600_AK073109	
RICEGENE29807	198.5	466.9	1046.7	217.0	98.4	111.7	89.0	100.6	16.1	15.1	Late embryogenesis abundant (LEA) group 1 family protein. [Os04t0589800-01]	Os04g0589800_AK063682	
RICEGENE12551	22.1	179.9	43.8	21.1	11.3	15.1	17.7	33.9	8.0	11.3	Late embryogenesis abundant (LEA) group 1 family protein. [Os06t0110200-01]	Os06g0110200_AK107973	
RICEGENE25662	16.2	125.3	6.9	8.1	11.1	12.8	15.5	15.9	51.3		Late embryogenesis abundant (LEA) group 1 family protein. [Os06t0324400-01]	Os06g0324400_AK063726	
RICEGENE23212	38.3	177.9	12.6	7.5	9.8	12.1	13.3	13.2	14.2		Late embryogenesis abundant (LEA) group 1 family protein. [Os08t0327700-01]	Os08g0327700_AK107930	
RICEGENE16252	2974.7	4520.0	10863.8	1962.6	327.6	266.6	655.4	257.4	94.2	118.3	Late embryogenesis abundant protein repeat containing protein. [Os01t0705200-01]	Os01g0705200_AK073837	
RICEGENE20659	23.7	212.1	65.6	17.6	11.3	209.4	14.5	37.5		140.9	Late embryogenesis abundant protein repeat containing protein. [Os03t0168100-01]	Os03g0168100_AK121575	
RICEGENE16505	197.3	117.4	176.7	30.6	31.0	17.8	67.4	17.0		3.9	Late embryogenesis abundant protein repeat containing protein. [Os02t0250600-01]	Os02g0250600_C1479812	
RICEGENE11289	8068.1	6917.7	3970.1	323.3	696.9	479.7	3255.7	46.4		261.4	LEA-like protein. [Os05t0542500-02]	Os05g0542500_AK119713	
Peroxidase													
RICEGENE05220	687.2	380.9	599.6	435.5	2291.9	1542.5	1325.2	1442.2	0.3	0.1	Similar to Peroxidase BP 1 precursor. [Os01t0963000-04]	Os01g0963000_AK104277	
RICEGENE02142	460.9	238.2	408.3	327.1	1507.3	987.2	860.3	956.3	0.4		Similar to Peroxidase BP 1 precursor. [Os01t0963000-01]	Os01g0963000_AK061131	
RICEGENE11966	20.8	6.7	14.6	9.2	22.9	61.6	25.9	29.0	0.5	0.4	Peroxidase (EC 1.11.1.7). [Os10t0109600-01]	Os10g0109600_AK061206	
RICEGENE11639	348.1	48.2	81.3	47.5	298.8	30.1	182.8	56.1		0.4	Similar to Class III peroxidase 136. [Os12t0111800-00]	Os12g0111800_C1552210	

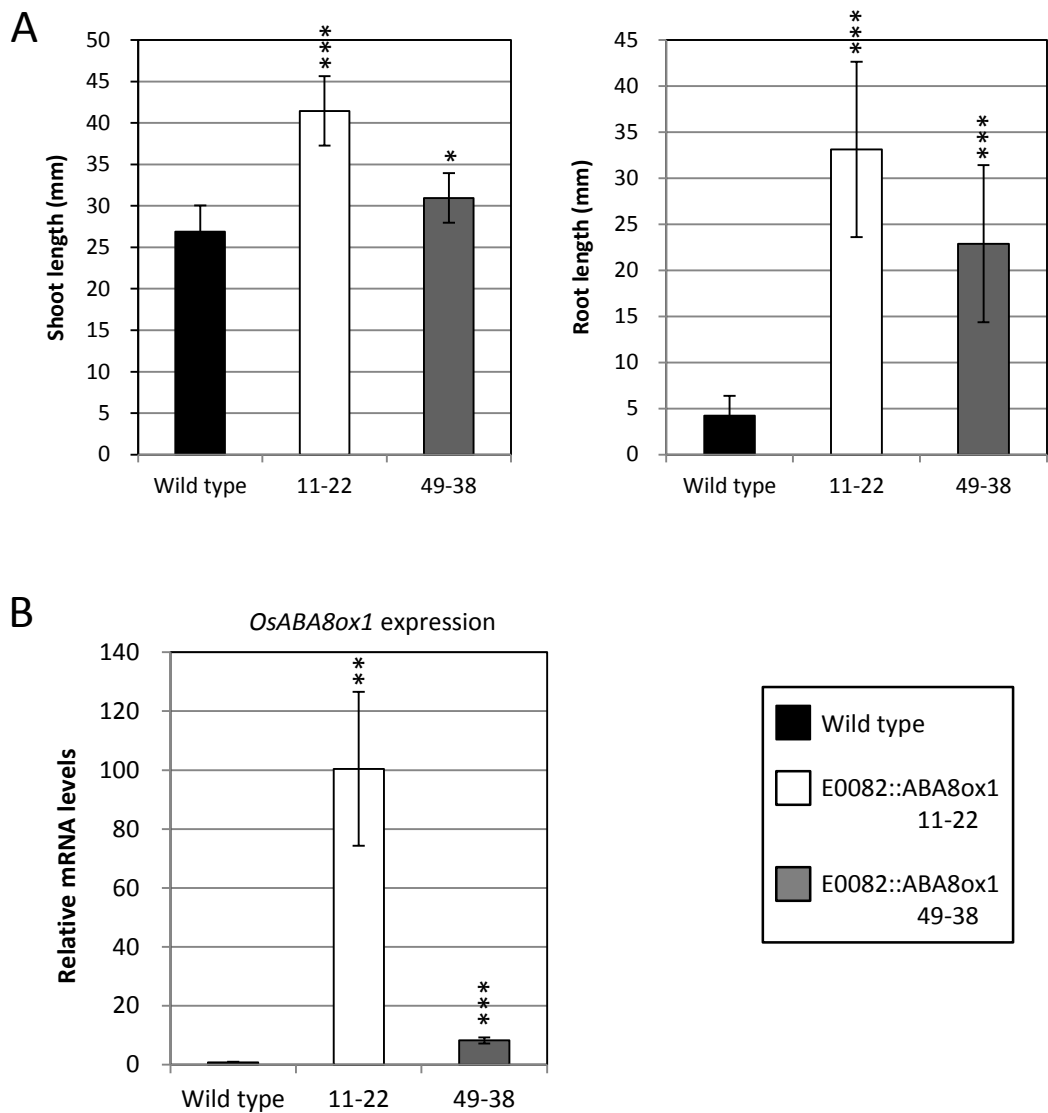
RICEGENE15706	168.8	378.5	12.5	320.4	393.0	2732.4	20.2	1300.3	0.5	0.3	Similar to Class III peroxidase GvPx2b (Fragment). [Os02t0240300-01]	Os02g0240300_AK104645
RICEGENE35278	7.0	326.3	8.2	227.1	344.9	2355.5	12.5	1139.4	0.4		Similar to Class III peroxidase GvPx2b (Fragment). [Os02t0240300-01]	Os02g0240300_AK067000
RICEGENE08929	7.6	6.3	20.5	8.5	10.4	106.7	18.5	14.1	0.3	0.1	Haem peroxidase family protein. [Os06t0521500-01]	Os06g0521500_AK061144
RICEGENE17013	2295.9	2069.0	1372.2	834.4	4948.1	3751.8	3500.3	1745.8	0.3	0.0	Similar to Class III peroxidase GvPx2b (Fragment). [Os06t0546500-01]	Os06g0546500_AK073833
RICEGENE24991	16.2	17.5	17.2	9.3	77.9	14.7	14.8	21.8	0.5	0.5	Haem peroxidase, plant/fungal/bacterial family protein. [Os07t0104500-01]	Os07g0104500_AK108824

Reference

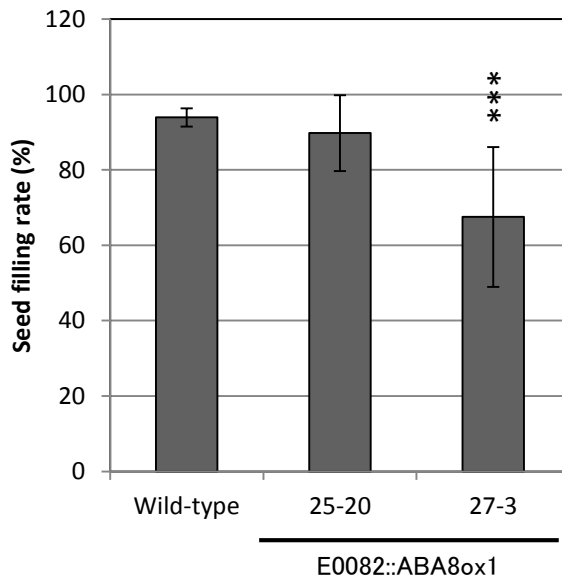
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- 2.BMC Genomics 2008, 9:550
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- 6.The ScientificWorld Journal Volume 2013, Article ID 397401, 9 pages



Supplementary Figure S2. Expression of *OsABA8oxs*, *OsNCEDs*, *OsABA2*, and *OsAAOs* under long-term cold stress. Rice seedlings after imbibition were transferred to and grown hydroponically at 15° C for 15 d. Values are means ± SD of three biological replicates. Significant differences between treated and control seedlings were determined by Student's *t*-tests. The letters a1, a2, and a3 represent $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, between day 3 at 15° C and day 1 at 25° C. The letters b1, b2, and b3 represent $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, between days 6 and 9 at 15° C and day 2 at 25° C. The letters c1, c2, and c3 represent $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, between days 12 and 15 at 15° C and day 3 at 25° C group. The letters d1, d2, and d3 represent $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, between day 15 at 15° C and day 4 at 25° C.



Supplementary Figure S3. Growth and *OsABA8ox1* gene expression of E0082::*ABA8ox1* 11-22 and E0082::*ABA8ox1* 49-38. Gene expression values are means \pm SD of three biological replicates. Significant differences between transgenic and wild-type seedlings were determined by Student's *t*-tests. ** and *** represent $P < 0.01$ and $P < 0.001$, respectively.

A**B**

Supplementary Figure S4. Seed fertility rate of E0082::ABA8ox1 25-20 and E0082::ABA8ox1 27-3. Typical images (A) and seed fertility rate (B) of wild-type, E0082::ABA8ox1 25-20, and E0082::ABA8ox1 27-3. Twenty rice plants were grown in soil in a greenhouse for 4 months (14 h light, 25° C; 10 h dark, 20° C). Seeds were harvested from completely ripened plants. The number of filling and empty seeds was counted, and the filling seed rate was calculated. The experiments were repeated two times. Significant differences relative to the wild type were determined by Student's *t*-tests. ***, $P < 0.001$.