

Supplementary Tables and Figures

Supplementary Table S1. Primers used in this study.

Name	Forward primer	Reverse primer	Purpose
<i>Ghd2</i> -OE	<i>ggggtacc</i> CTGGGAGATTTCTTCT GTTT	<i>cgggatcc</i> TCGAGTCCATGAGGC ATA	Overexpression
<i>Ghd2</i> -flag	<i>ggggtacc</i> CTGGGAGATTTCTTCT GTTT	<i>cgggatcc</i> GGTGACGGCGCACGG GGC	Overexpression (3×flag)
<i>Ghd2</i> -sub	<i>cgcgatcc</i> CTGGGAGATTTCTTCT GTT	<i>cgcgatcc</i> CTTGACGAACCGGC CCTT	Subcellular localization
<i>Ghd2</i> -grna	<i>ggca</i> ATCCTCATCGTGCTTCAAG G	<i>aaac</i> CCTTGAAGCACGATGAG GAT	CRISPR
<i>Ghd2</i> -cas-P CR	TTTGTGCTGGGAGATTTCTT TTTGTGCTGGGAGATTTCTT	GCCTCGTAGTTGAGCCTGA	PCR for sequencing
<i>Ghd2</i> -cas-s eq	TCGGTGGAGGAGGAGGCG		Sequencing
<i>CAS9</i> -PCR	GCATGAAGAGGATCGAGGAG	GATCTCTTGCTCGGACTTGG	<i>CAS9</i> detection
<i>HPT</i> -PCR	TACACAGCCATCGGTCCAGA	TAGGAGGGCGTGATATGTC	<i>HPT</i> detection
<i>Ghd2</i> -probe	ATGGTCGAAGCGCAA	ACAACCCCGAGTAATGG	Northern
<i>Ghd2</i> -BiFC	<i>cgcgatcc</i> CTGGGAGATTTCTTCT GTT	<i>ggggtacc</i> GGTGACGGCGCACGG GGC	
GF14b-BiFC C	<i>cgggatcc</i> GGCATTGTAGAGTTT TTAGAT	<i>cgggatcc</i> CTGCCCTCGCTGGA GT	
GF14c-BiFC C	<i>cgggatcc</i> TAATCCCTTAATTGGT CAAA	<i>cgggatcc</i> CTGCCCTCGCAGGC GT	BiFC
OsARID3- BiFC	<i>cgggatcc</i> GCATCTGGATTTGAGC CTA	<i>cgggatcc</i> CATTAACCTTTGACTGC TCGAA	
<i>Ghd2</i> -Y2H N1	<i>attB1</i> -TGAGCTGCAGCTCGGAGA A	<i>attB2</i> -TCGAGTCCATGAGGCAT ACG	
<i>Ghd2</i> -Y2H- N2	<i>attB1</i> -TGAGCTGCAGCTCGGAG AA	<i>attB2-ccta</i> CTCCTCCACCGACGC C	
<i>Ghd2</i> -Y2H- N3	<i>attB1</i> -TGAGCTGCAGCTCGGAG AA	<i>attB2-ccta</i> TGCGGCCGCCGCGT CATC	
<i>Ghd2</i> -Y2H- N4	<i>attB1</i> -TGAGCTGCAGCTCGGAG AA	<i>attB2-ccta</i> ATCCGGGAGCTGCT GCAC	
<i>Ghd2</i> -Y2H- N5	<i>attB1</i> -TGAGCTGCAGCTCGGAG AA	<i>attB2-ccta</i> CAGCTCGTTGCTGTC GTC	Y2H
<i>Ghd2</i> -Y2H- N6	<i>attB1</i> -TGAGCTGCAGCTCGGAG AA	<i>attB2-ccta</i> GCTGTTGGAGGCAA CACC	
<i>Ghd2</i> -Y2H- C1	<i>attB1</i> -TGAGCTGCAGCTCGGAG A	<i>attB2-ccta</i> CACCGTCATCATCCC CAC	
<i>Ghd2</i> -Y2H- C1	<i>attB1</i> -TGGGGATGATGACGGTG A	<i>attB2</i> -TCGAGTCCATGAGGCAT ACG	

Ghd2-Y2H-C2	<i>attB1</i> -GGGTATCTCTCCCATCAT	<i>attB2</i> -TCGAGTCCATGAGGCAT ACG	
Ghd2-Y2H-C3	<i>attB1</i> -TGCAGGACTCGTTCTACA	<i>attB2</i> -TCGAGTCCATGAGGCAT ACG	
Ghd2-Y2H-C4	<i>attB1</i> -GCTTTGTCAACTTCGAAC	<i>attB2</i> -TCGAGTCCATGAGGCAT ACG	
Ghd2-Y2H-C5	<i>attB1</i> -CCTCATCGTGCTTCAAGG	<i>attB2</i> -TCGAGTCCATGAGGCAT ACG	
Ghd2-Y2H-C6	A	<i>attB2</i> -TCGAGTCCATGAGGCAT ACG	
<hr/>			
Ghd2-none	<i>cgggatcc</i> CTGGGAGATTTCTTCT GTTT	<i>ggggatcc</i> TCGAGTCCATGAGGC ATA	
Ghd2-gal4 BD	<i>cgggatcc</i> AGCTGCAGCTCGGAGA AG	<i>ggggatcc</i> TCGAGTCCATGAGGC ATA	
ICL-190	<i>cccaagctt</i> GAAACGAGAACTACG ACGAGC	<i>gaagatct</i> CACGCACTGTCAACG ATGAG	
MS-190	<i>cccaagctt</i> TCTGTGAGACAGATAG GTGGA	<i>gaagatct</i> GTGTGAGTAGAAAAT GGGAGT	
<i>OsPPDKB</i> -190	<i>gaagatct</i> CCGTGTTTCACTGTTTC CCAATGTC	<i>gaagatct</i> CGATCTCCCCTCCAAC TCCAAGC	LUC assay in protoplasts
<i>OsAS1</i> -190	<i>cccaagctt</i> GACAAATACGCTGAA TGCATA	<i>gaagatct</i> GCCGCGAGGATTACT ACC	
<i>OsSGR</i> -190	<i>cccaagctt</i> CGCTCTCGTTCATCCT GTT	<i>gaagatct</i> GCCTAACAACCAAAA CGACTC	
<i>OsNYC3</i> -190	<i>cgggatcc</i> AATCGTAGACCTCAAA ACCC	<i>cgggatcc</i> CAGGAAGAAAAAAA GACATAGC	
<i>OsPAO</i> -190	<i>cccaagctt</i> GCCTGCACCAGGGTA AAG	<i>gaagatct</i> GAAGAAGAGAGTAGG AAACGGA	
<hr/>			
<i>Ghd2</i> -qRT	TTGCCTCCAACAGCAGGG	GCTTCGGATGAGCGCG CCTCTGAATAATCCTCATCAC	
ICL-qRT	GACAGGGCCTGGAAGTGAGA ACTTCTCCGAGTACTTTGCTCA	ATCCT TCTAGTGCAAACAAACAGGA	
MS-qRT	TACTT	ATTGT	
<i>OsAS1</i> -qRT	TGTGCCGTTCTCGACAA	TTCCACTCAGGGTCCATGCT	
<i>OsSGR</i> -qRT	TTCGTTTGGTTGCCATGGTA	AGCACGCAGCGTCATTTG	qPCR
<i>OsNYC1</i> -qRT	GTCGTCTGCGCATTCATAATTC	GGGATCATGTGCCTGGAAGA	
<i>OsNYC3</i> -qRT	TGTGTGCTCCAAAGGGACAA	CCCTGCCTTTGGCACCTA	
<i>Ubi</i> -qRT	AACCAGCTGAGGCCCAAGA	ACGATTGATTTAACCAGTCCA TGA	

Supplementary Table S2. Public microarray results of *OsK*, *OsL*, and *OsJ* in response to drought stress treatment at 7-day-old seedling stage.

Name	Normal growth			Drought stress		
	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
<i>OsK</i>	277.1	264.6	332.5	154.2	139.7	155.4
<i>OsL</i>	156.8	185.6	209.3	15.2	18.5	20.9
<i>OsJ</i>	828.5	780	1075	373	319.9	360

Supplementary Table S3. Public microarray results of *OsK* in response to drought stress treatment in three rice organs.

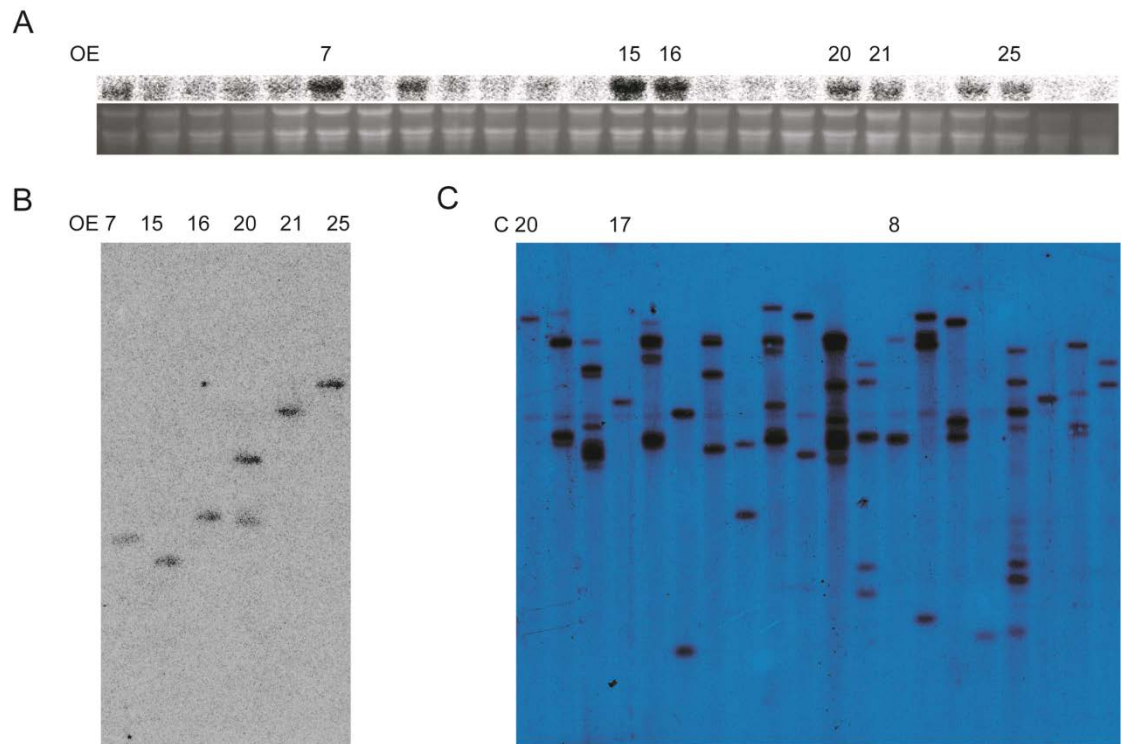
Organ	Drought-1		Drought-2		Drought-3	
	LogRatio	<i>P</i> -value	LogRatio	<i>P</i> -value	LogRatio	<i>P</i> -value
Flag leaf	-0.317	2.35E-02	-0.233	1.58E-01	-0.805	2.15E-02
Shoot	-0.242	2.79E-01	-0.016	8.49E-01	-0.975	1.10E-04
Panicle	N/A	N/A	N/A	N/A	-1.221	3.58E-02

Supplementary Table S4. GO analysis of up-regulated DEGs in the *Ghd2*-OE plants during drought stress treatment.

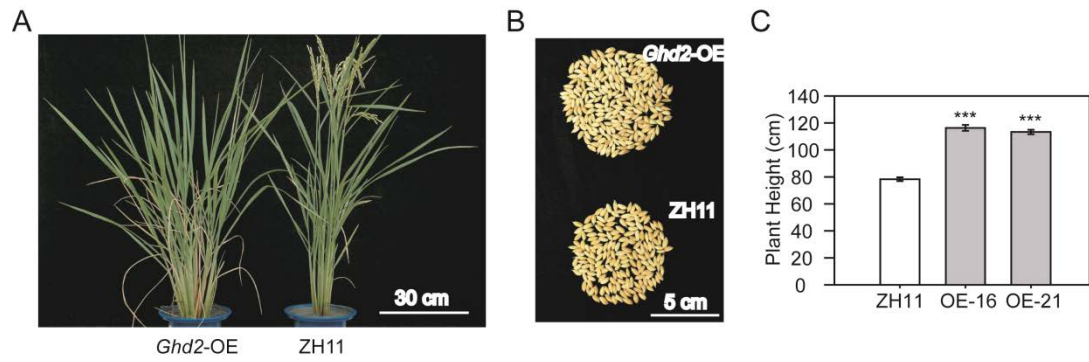
GO Term	Count ^a	P-value
Biological process		
GO:0003333 amino acid transmembrane transport	36	1.20E-06
GO:0098656 anion transmembrane transport	65	3.10E-06
GO:0006787 porphyrin-containing compound catabolic process	25	3.70E-06
GO:0033015 tetrapyrrole catabolic process	25	3.70E-06
GO:0051187 cofactor catabolic process	28	6.70E-06
GO:0015849 organic acid transport	129	3.80E-05
GO:0046942 carboxylic acid transport	129	3.80E-05
GO:0015996 chlorophyll catabolic process	23	6.60E-05
GO:0046149 pigment catabolic process	24	7.90E-05
GO:0006865 amino acid transport	75	8.90E-05
Molecular function		
GO:0015171 amino acid transmembrane transporter activity	71	6.60E-05
GO:0005342 organic acid transmembrane transporter activity	105	7.50E-05
GO:0046943 carboxylic acid transmembrane transporter activity	105	7.50E-05
GO:0015293 symporter activity	126	0.00023
GO:0008514 organic anion transmembrane transporter activity	131	0.0003
GO:0015294 solute:cation symporter activity	37	0.00045
GO:0016746 transferase activity, transferring acyl groups	344	0.00056
GO:0008509 anion transmembrane transporter activity	200	0.00078
GO:0016798 hydrolase activity, acting on glycosyl bonds	363	0.00087
GO:0016747 transferase activity, transferring acyl groups other than amino-acyl groups	308	0.00089
Cellular component		
GO:0000329 fungal-type vacuole membrane	14	0.00015
GO:0000324 fungal-type vacuole	15	0.00019
GO:0009570 chloroplast stroma	331	0.00024
GO:0009532 plastid stroma	335	0.00026
GO:0000322 storage vacuole	17	0.00028
GO:0044434 chloroplast part	651	0.00151
GO:0009536 plastid	1784	0.00202
GO:0044435 plastid part	686	0.0024
GO:0009507 chloroplast	1755	0.00349
GO:0000323 lytic vacuole	88	0.00478

Note: The listed terms is top ten overrepresented GO terms according to the *P*-value.

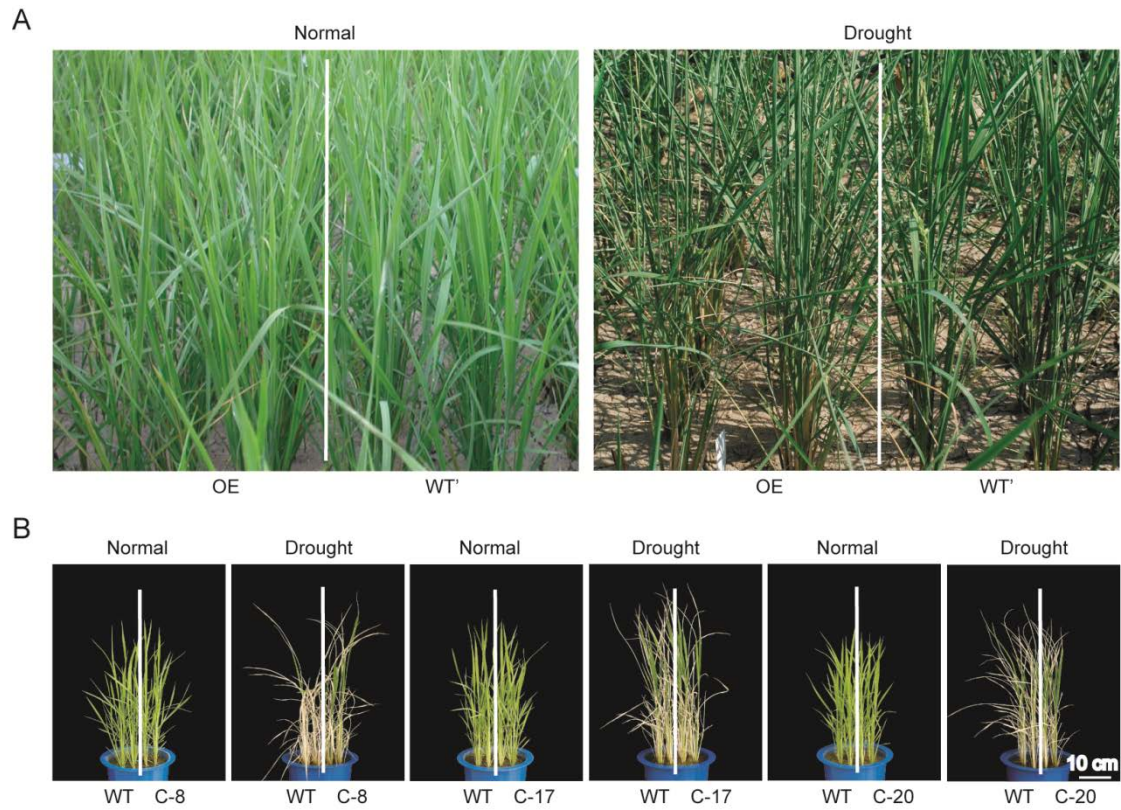
^a The number of differential expressed genes fall in each GO in the *Ghd2*-overexpressing lines.



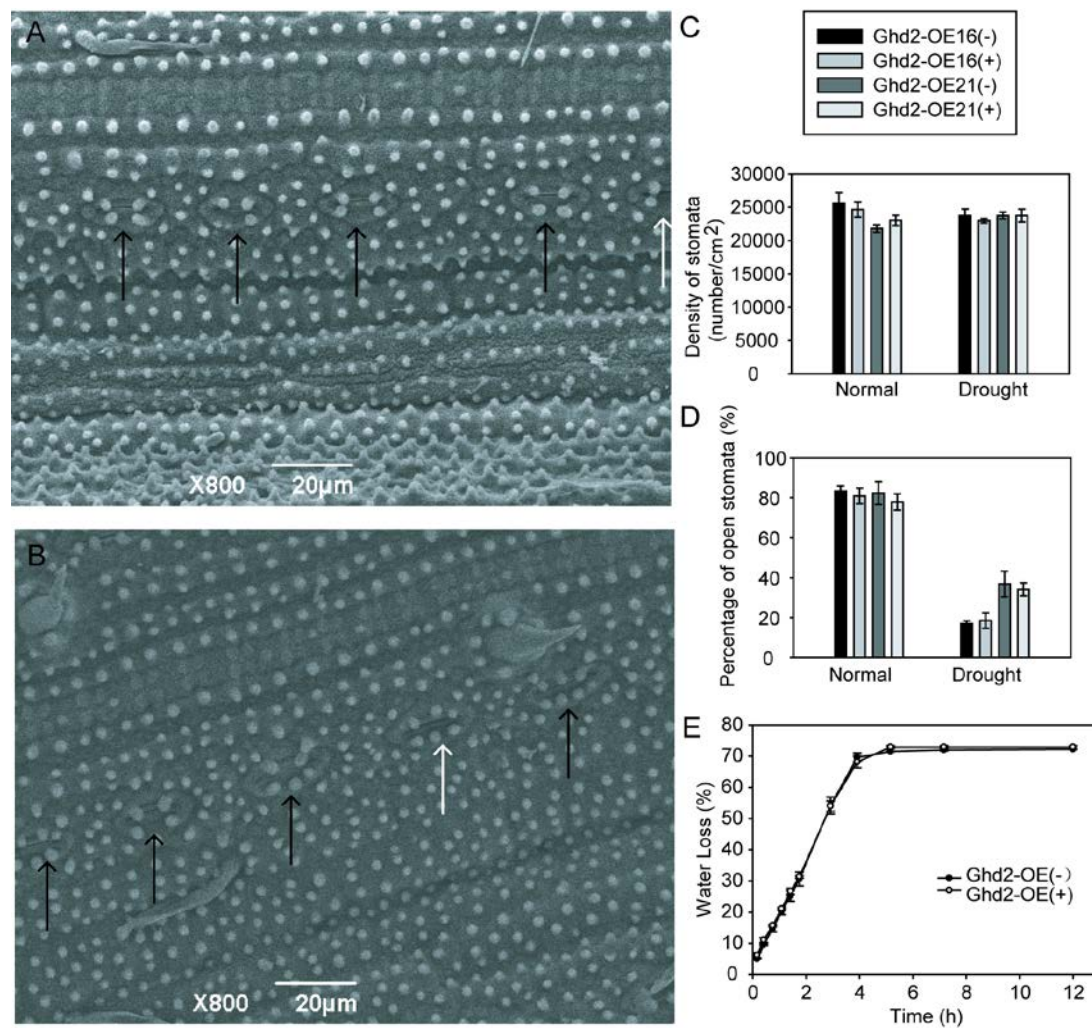
Supplementary Figure S1. Expression level and copy number detection by Northern and Southern blot respectively. Southern blot analysis used hygromycin resistance gene as a probe. (A) The *Ghd2*-OE lines were identified by Northern blot analysis. (B) Copy number of the *Ghd2*-OE lines detected by Southern blot analysis. (C) Copy number detection of the *Ghd2*-CRISPR lines by Southern blot analysis. Probes of (A) and (B) were radio-labeled, and probe for (C) was digoxigenin-labeled.



Supplementary Figure S2. Phenotypes of the *Ghd2*-OE plants with respect to grain number, plant height, and heading date. (A) Phenotypes of the *Ghd2*-OE and ZH11 plants on heading date. (B) Phenotypes of the *Ghd2*-OE and ZH11 plants on grain number per main panicle. (C) Plant height of the *Ghd2*-OE and ZH11 plants. Data represent the mean \pm SE (n=9). *** P <0.005, t -test.



Supplementary Figure S3. Phenotypes of the *Ghd2*-OE plants at 60 DAG and the *Ghd2*-CRISPR plants at the seedling stage under drought stress treatment. (A) Phenotypes of the *Ghd2*-OE21 plants at 60 DAG under drought stress treatment in the field. (B) Phenotypes of the *Ghd2*-CRISPR plants at the seedling stage under drought stress treatment.



Supplementary Figure S4. Stomata and water loss rate in the *Ghd2*-OE and WT' plants. (A-B), SEM images of the *Ghd2*-OE (A) and WT' (B) plant leaves under drought stress. The open and closed stomata are indicated by white and black arrows respectively. (C-D), Stomata density (C) and percentage of open stomata (D) in the overexpression (*Ghd2*-OE (+)) and WT' control (*Ghd2*-OE (-)) plant leaves under drought stress. (E) Water loss rate in detached rice leaves of the *Ghd2*-OE and WT' control plants (n=3).

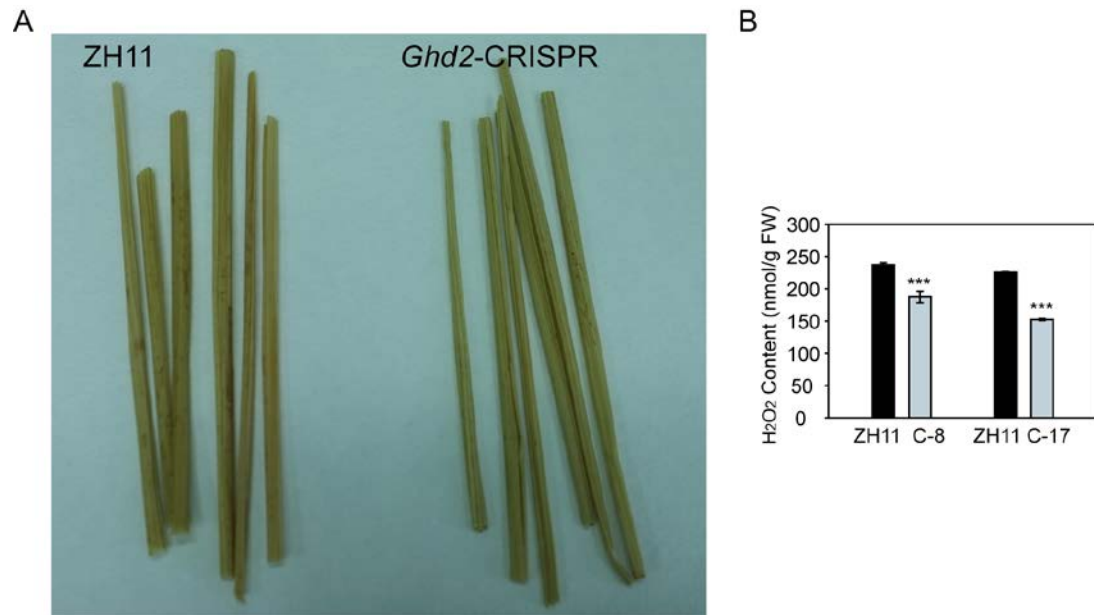
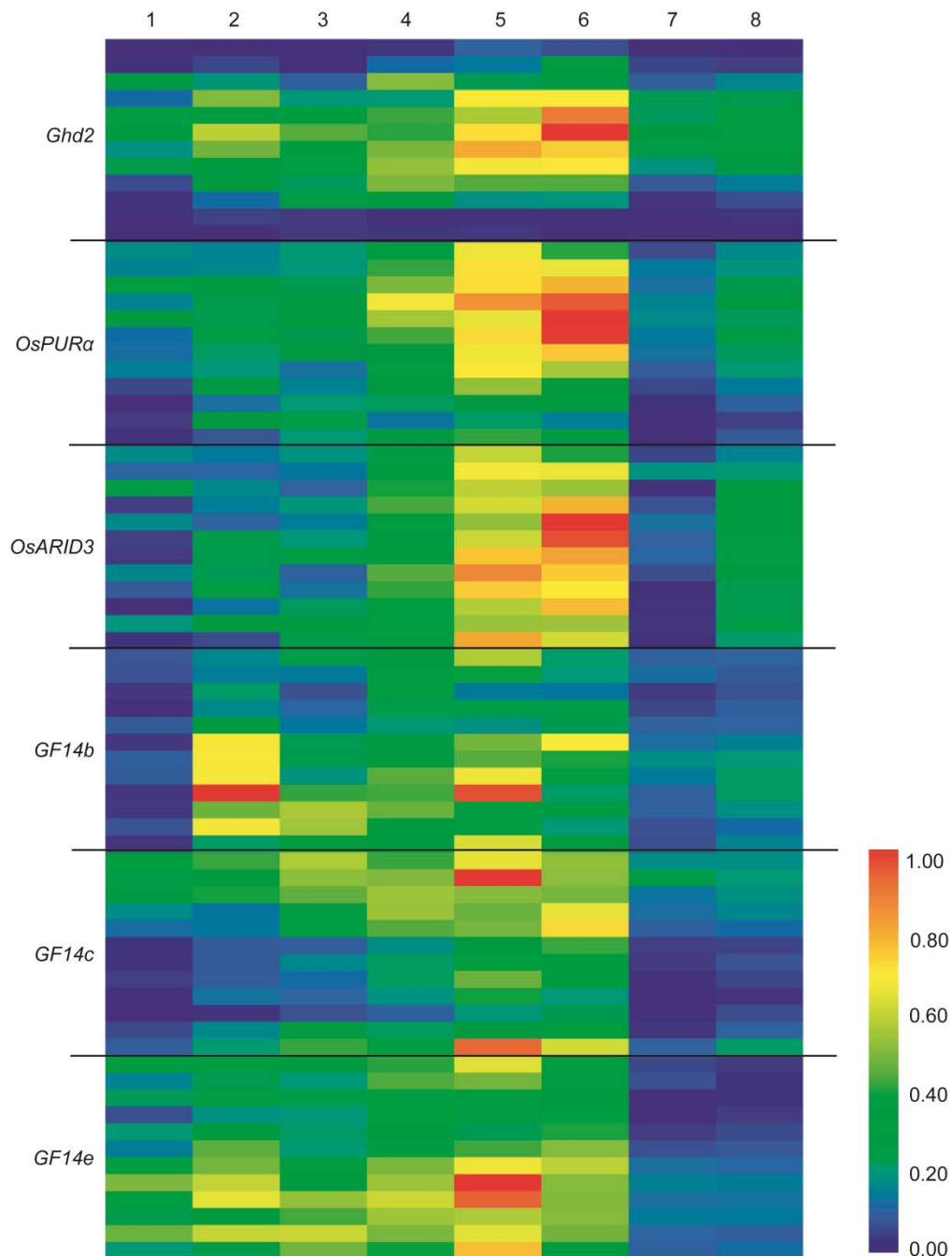
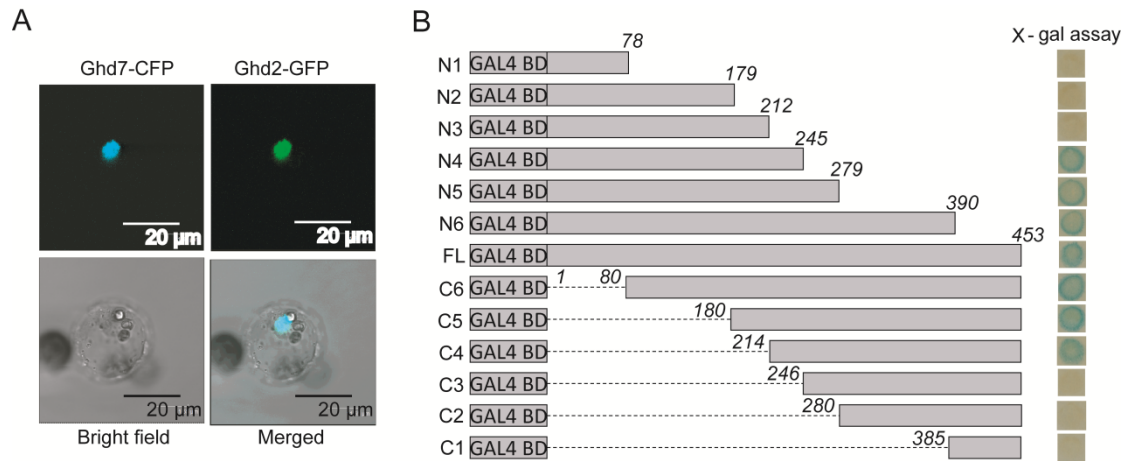


Figure S5. H₂O₂ content in the *Ghd2*-CRISPR and WT plants under the drought stress treatment. (A) DAB staining of leaves in the *Ghd2*-CRISPR and ZH11 plants during the drought stress. (B) H₂O₂ content measurement in the leaves of the *Ghd2*-CRISPR lines (C-8 and C-17) and ZH11 plants during the drought stress. *** $P < 0.005$, t -test. FW, Fresh Weight.



Supplementary Figure S6. Diurnal expression levels of *Ghd2*, *OsPURA*, *OsARID3*, and *14-3-3s* in rice leaves at different developmental stages. Numbers from 1 to 8 in the *x*-axis refer to “vegetative 1”, “vegetative 2”, “vegetative 3”, “vegetative reproductive”, “reproductive 1”, “reproductive ripening”, “ripening 1”, “ripening 2”, respectively (as named in the website), and the *y*-axis indicates time points of each gene from am10:00 to am 8:00. Original data was obtained from rice expression database (<http://ricexpro.dna.affrc.go.jp/>) and analyzed using Heatmap Illustrator.



Supplementary Figure S7. Ghd2 is located in the nucleus and functions as a transcriptional activator. (A) Nuclear localization of Ghd2 in rice protoplast. Nuclear localized CCT domain containing protein Ghd7 was used as a maker to show the nucleus. (B) The activation assay of a series of deletion mutants of Ghd2 in yeast to show the transactivation activity.