Supporting Information

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SI Methods

Plant Growth and Treatments. For analysis of gene expression levels under various stresses, seeds of *japonica* rice Zhonghua 11 were germinated and grown in sandy soil for 4 wk. The 4-wk-old seedlings were treated with cold stress (exposing plants to 4 °C), oxidative stress (20 μM methyl viologen), and phytohormones (100 μM abscisic acid, 100 μM jasmonic acid, 50 μM indole-3-acetic acid, 1 mM ethephon, or 100 μM gibberellic acid), and leaves were sampled for a designated time. For analysis of the tempospatial expression of *DWA1*, tissues or organs, such as the leaf, root, sheath, stem, panicle, and spikelet, were sampled during the whole life cycle of rice.

After drought treatment, the fully rolled top-second leaves of 4-wk-old plants (WT, mutant, complementation, and overexpression rice) were sampled for SEM, fatty acid (FA), and wax measurement, as well as expression level analysis of wax-related genes. The top-second leaves of 4-wk-old plants without drought stress were sampled as normal controls for these analyses.

The drought testing at reproductive stage was carried out essentially as described by Yue et al. (1) with minor modifications. Plants grown in PVC pipes at the booting stage were subjected to drought stress by draining out the water in the pipes. When the leaves became fully rolled (with relative water content in leaves of 70–75%), as observed at noon for the first time, the plants were rewatered for recovery, and this was defined as moderate drought stress. Severe drought was applied by keeping the fully rolled leaf state for 1 wk, followed by recovery. Drought treatment was given for only one cycle in this study.

Identification of the Transfer-DNA Insertion. The genomic DNA fragment flanking the left border of the transfer-DNA (T-DNA) insertion in the d*rought-induced wax accumulation 1* (*dwa1*) mutant was isolated by thermal asymmetric interlaced (TAIL)-PCR (2). The tagged flanking sequence was searched against the Rice Annotation Database (http://rice.plantbiology.msu.edu/), which resulted in a match to the *DWA1* gene. A pair of *DWA1*-specific primers (EL39F/R) and the T-DNA left border primer (NTLB5, as a vector primer) was combined to genotype the mutant families and functional complementation plants.

Constructs and Rice Transformation. An 8.5-kb genomic fragment of *DWA1* gene, including a 134-bp 5′-UTR fragment and the full-length *DWA1* gene region, was amplified from rice genomic DNA with primer pairs DWA1FLF/R (EcoRV restriction site added as an adapter) using long-distance (LD)-PCR. LD-PCR was performed in a 50-μL volume as follows: one cycle at 95 °C for 2 min; 32 cycles at 98 °C for 15 s and 68 °C for 8.5 min, with a 15-s increase to the extension time for each cycle of the last 10 cycles; and the final extension at 68 °C for 12 min. For making the functional complementation construct, the full-length *DWA1* genomic fragment was digested with EcoRV (blunt end) and cloned into pCAMBIA1301U-NPTII (G418 resistance) vector (digested by SmaI, blunt end) in front of a maize *Ubiquitin* promoter. This complementation construct was introduced into homologous *dwa1* mutant by *Agrobacterium*-mediated transformation (3). To generate the *DWA1*-overexpression construct, the full-length *DWA1* genomic fragment was digested with EcoRV (blunt end) and inserted into pCAMBIA1301U-HPT (hygromycin resistance) vector (digested by SmaI, blunt end) in front of the constitutive maize *Ubiquitin* promoter. This overexpression construct was introduced into *japonica* rice ZH11 by *Agrobacterium*-mediated transformation.

Expression Level Analysis. For real-time quantitative RT-PCR analysis, first-strand cDNA was synthesized by SuperScript III reverse transcriptase (Invitrogen). PCR was performed on a 7500 Real-Time PCR System (Applied Biosystems) using the SYBR Green PCR Kit (Takara) in a reaction volume of 20 μL. The thermal cycler was set as follows: 95 °C for 10 s, 50 cycles of 95 °C for 5 s, and 60 °C for 34 s. The rice *Osprofilin1* gene (LOC_Os06g05880) was used as the reference gene because of its highly stable expression level in different rice tissues and under various growth conditions (4). The relative expression levels were determined by the methods described previously (5).

For the promoter-GUS reporter assay, the promoter region of *DWA1* was amplified from rice genomic DNA by PCR with a pair of primers, *DWA1*ProF/R. The amplified DWA1 promoter was digested with HindIII/BamH and then cloned into the corresponding restriction site of the pDX2181 vector, which contains a GUS reporter gene to generate pDX2181-*DWA1*pro::GUS. The recombinant plasmid was transformed into rice Zhonghua 11 via *Agrobacterium*-mediated genetic transformation. Homozygous transgenic lines were selected for histochemical analyses. Histochemical GUS staining was performed as described previously (6).

Various tissues and organs of the pDX2181-*DWA1*pro::GUS transgenic plants at the reproductive stage under normal conditions were collected for GUS staining. Leaves from moderately drought-stressed 4-wk-old plants were also sampled for GUS staining. For a more detailed analysis of the expression of *DWA1* by GUS staining, cross-sections (10 μm in thickness) of the samples were observed with a light microscope. Semithin sections (2 μm) were also prepared and observed by means of a Leica RM2265 microtome. The sections were photographed using a differential interference microscope (Nikon Eclipse 80i).

Prediction of putative *cis*-elements was performed using Signal Scan Search against the plant cis-acting regulatory DNA elements (PLACE) database (7). A 2-kb sequence upstream of *DWA1* ATG initiation codon was selected as the promoter region for this analysis.

Southern Blot Analysis. The copy number of the integrated T-DNA in the rice genome was determined using Southern blot analysis. Genomic DNA was extracted from rice leaves using the hexadecyl trimethyl ammonium bromide (CTAB) method, digested with EcoRI and HindIII, separated in a 0.8% agarose gel, and then transferred onto a nylon membrane. The hybridization was performed using the hygromycin resistant gene (*Hpt*) as the probe.

Quantification of Epidermal Traits. To quantify the leaf water loss rate, samples from the top-second leaves were detached from 4-wk-old seedlings. The leaves were dehydrated, and measurements were performed at room temperature. Three replicates were included for each sample in the experiment.

Epidermal permeability was measured using the chlorophyll leaching assay. Fully expanded leaves of 4-wk-old plants were collected, and the fresh weight of each leaf was measured immediately. Leaf samples were immersed in 30 mL of 80% (vol/vol) ethanol in 50-mL conical tubes at room temperature. Aliquots of 100 μL of the supernatant were removed from the solution at 10-min intervals (up to 60 min) and 120 min after immersion. The content of the extracted chlorophylls was determined by measuring the absorption spectra at 647 and 664 nm using a DU640 spectrophotometer (Beckman) and calculated as described pre-

viously (8). Extracted chlorophyll contents at the individual time points were displayed as the chlorophyll leaching rate in the leaf tissues.

Leaf cuticle features of the *dwa1* mutant and WT were observed by SEM. Segments of the top-second (fully expanded) leaves were fixed in 2.5% (vol/vol) glutaraldehyde and dried. Samples were sputter-coated with gold particles for 15 min using a JEOL JFC-1600 AUTO FINE COATER. Coated surfaces were viewed using a JEOL JSM-6390LV scanning electron microscope.

FA and Epicuticular Wax Quantification. FA extraction was performed as described by Xia et al. (9). Wax extraction was conducted according to the method of Lü et al. (10) with some modifications. Fully expanded leaves (200–500 mg) of 4-wk-old plants were immersed in 30 mL of gas chromatography grade hexane for 30 s at 67 °C. Fifty micrograms of *n*-tetracosane (Fluka) and nonadecanoic acid (Fluka) were added to the extracted hexane solvent as internal standards. The hexane was then evaporated by a nitrogen evaporator (Dry N-EVAPTM111; Organomation Associates, Inc.). After the samples were transferred to GC vials, 40 μL of N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (Fluka) and 40 μL of pyridine (Sigma) were added and the samples were derivatized at 100 °C for 30 min. BSTFA and pyridine were then removed under the stream of nitrogen. With 1,000 μL of hexane added, the samples were loaded for GC-MS analysis.

Sialylated samples were analyzed by capillary GC-MS (GCMS-QP2010Plus; Shimadzu; 30-m column, 5-ms Restek Rxi, 0.25-mm i.d., 0.25-μm df) using a helium carrier gas inlet pressure of 1.0 mL/min and a mass spectrometric detector (GCMS-QP2010Plus; Shimadzu). The column temperature was programmed as follows: injection at 220 °C and maintenance of the temperature at 220 °C for 2 min, followed by an increase to 290 °C at a rate of 3 °C per minute. The temperature was then maintained at 290 °C for 10 min, after which it was raised to 300 °C at a rate of 2 °C per minute and held for 10 min. Quantitative analysis of the mixtures was performed using capillary GC with a flame ionization detector under the same GC conditions described above. Single wax compounds were quantified against the internal standard by integrating the peak areas.

Enzymatic Activity Assay of DWA1 A Domain. The cDNA fragment encoding the DWA1 A domain (aa 427–1,093) was amplified using PCR primer pairs *DWA1*PE4F/R and inserted in-frame with GST tag in the bacterial expression vector pGEX-6P-1 (GE Healthcare). This construct, called pGEX6p-DWA1:AMP-binding domain (AMPbd), was transformed into *Escherichia coli* BL21 (DE3). The A domain expression was induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside for 24 h and shaken slowly at 18 °C to improve the yield of soluble and active recombinant protein.

For purification of DWA1 A domain, BL21 cell pellets were harvested by centrifugation (4000 × *g* for 15 min at 4 °C) of 750-mL cell cultures. The collected bacterial cells were resuspended in 40-mL cold (4 °C) 1× PBS [140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4 (pH 7.3)]. The resuspended cells were added with 1 mg/mL lysozyme (Sigma) and 1 mM DTT (Sigma), incubated on ice for 30 min, and then sonicated in an ice bath. Cell debris was removed by centrifugation (13000 × *g* for 10 min at 4 °C). The isolated supernatants were filtered through a 0.45-μm filter and then applied to an affinity chromatography column packed with 400 μL of Glutathione 4 Fast Flow (GE Healthcare) to purify the recombinant GST-tagged DWA1 A domain protein following the manufacturer's instructions. Sepharose-bound proteins were eluted with 50 mM Tris·HCl buffer (pH 8.0) containing 10 mM reduced glutathione and detected by 12% SDS/PAGE with Coomassie Brilliant Blue staining. GST protein purified from the bacteria with the pGEX-6P-1 empty vector was used as a control. Protein concentrations were determined according to the Bradford method (11), with BSA as a standard.

Enzymatic activity of the recombinant protein was determined using an enzyme-coupled colorimetric assay (12, 13) with modifications. Potassium salts for long or very long-chain FAs were prepared by mixing the FAs in hexane (HPLC grade; Sigma) with 0.01 M methanolic KOH, followed by evaporation of the solvent under nitrogen gas to minimize oxidation. The enzymatic assay solution contains 0.15 M 3-(*N*-morpholino)-propanesulphonic acid/NaOH (pH 7.6), 0.5 mM FA potassium salt, 0.5 mM CoA, 4.5 mM ATP, 12 mM $MgCl_2$, 1 mM DTT, 0.55 mM Triton X-100, 1% methanol, 0.5 unit of acyl-CoA oxidase (NJADFN), 1,000 units of catalase (Sigma), and 10 μL of the recombinant protein in a total volume of 50 μL. After incubation at 30 °C for 15 min, the reaction was stopped by addition of 50 μL of 2 M KOH. Then, 50 μL of 0.6% 4-amino-3-hydrazino-5-mercapto-1,2,4-tzole (Sigma) in 0.5 M HCl was added to the reaction solution. After a 10-min incubation at 37 °C, 200 μL of 0.75% KIO_4 was added. After dilution with 650 μL of double distilled (dd) H_2O, absorbance at 550 nm was measured at room temperature using a DU640 spectrophotometer (Beckman). The molar absorption coefficient, $\varepsilon = 29{,}200\ M^{-1}{\cdot}cm^{-1}$, of the resulting purple dye was used to calculate the amount of acyl-CoA synthesized.

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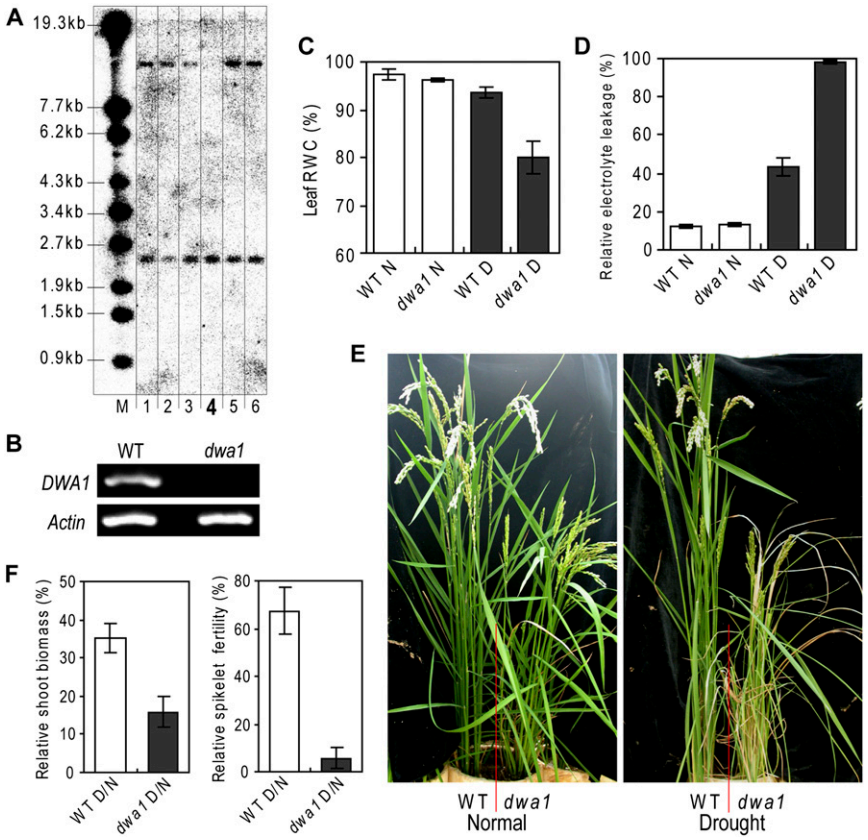

Fig. S1. Identification of the drought-hypersensitive *dwa1* mutant in rice. (*A*) T-DNA copy number analysis of *dwa1* mutant families by Southern blot; 8-25H (in lane 4) is a *dwa1* homozygous line with a single copy of T-DNA insertion used in this study. M, DNA marker EcoT14; lane 1, 8-2H; lane 2, 8-10H; lane 3, 8-11H; lane 4, 8-25H; lane 5, 14-1H; lane 6, 14-16H. (*B*) Detection of *DWA1* transcriptional level in the panicle of *dwa1* mutant and WT by semiquantitative RT-PCR. (*C*) Water loss in the leaves of WT and *dwa1* mutant indicated by leaf relative water content (RWC) measurement. (*D*) Electrolyte leakage measurement of WT and mutant seedlings under normal and drought treatments. (*E*) Mutant and WT plants were subjected to drought stress at the heading stage when grown in PVC pipes filled with sandy soil. (*F*) Relative spikelet fertility and shoot biomass of *dwa1* and WT after stress at the heading stage in a drought field with removable rain-off shelters.

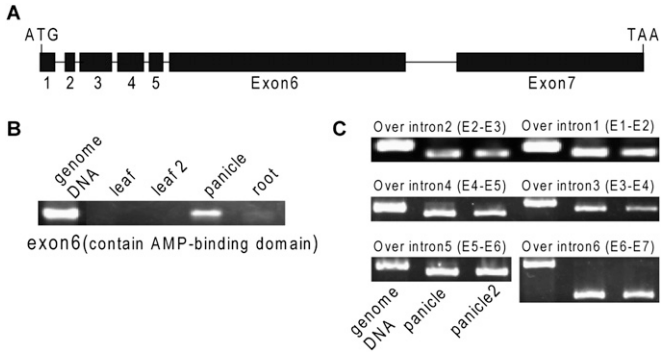

Fig. S2. Preliminary identification of transcription activity and gene structure of *DWA1*. (*A*) Predicted gene structure of *DWA1* in the genome database. (*B*) RT-PCR result in various tissues of rice plants showed the transcription activity of *DWA1* (exon6) in panicles. (*C*) Confirmation of the intron/exon structure of *DWA1* using transcription analysis of its exons in panicle tissues. E1–E7 indicates the primer pairs corresponding to exon1 to exon7, which were designed to cross over each intron. Genome DNA was used in PCR analysis for assisting the confirmation.

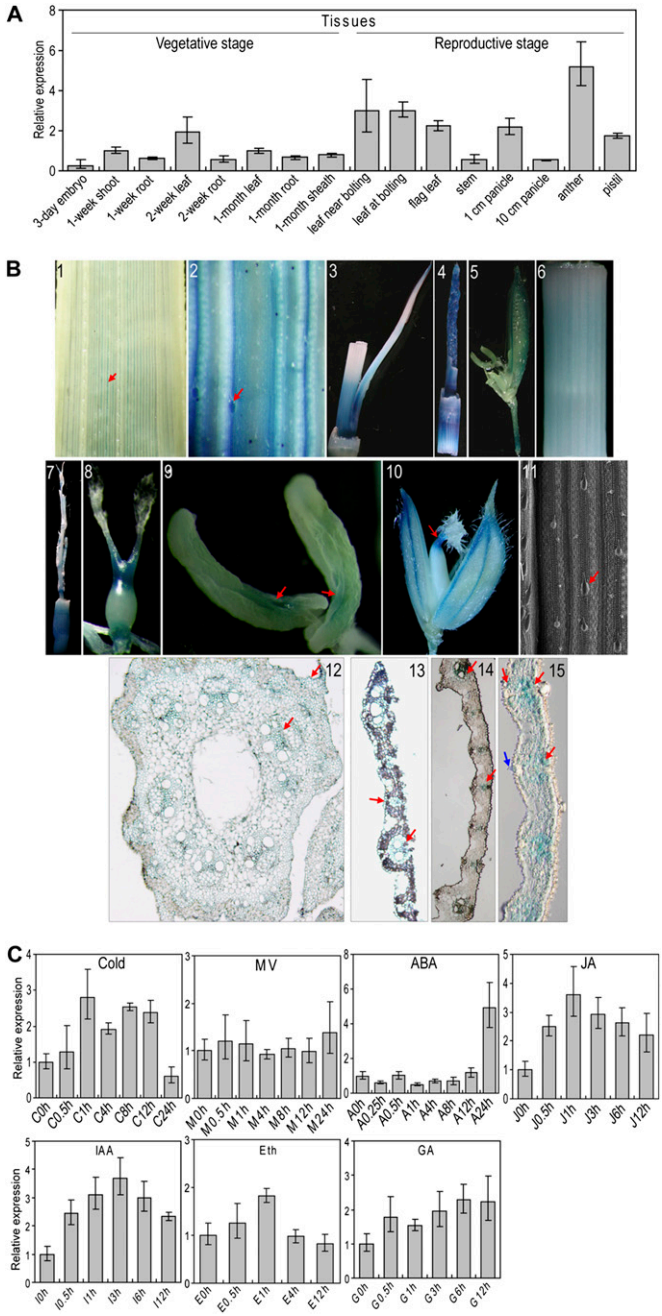

Fig. S3. Expression profiles of *DWA1* under normal and stress conditions. (*A*) Expression level of *DWA1* in various tissues. (*B*) *DWA1* promoter::GUS fusion expression patterns in transgenic rice plants under normal conditions. GUS staining was shown in mature leaves (1 and 2), axillary bud (3), young panicle (4), spikelet (5), stem (6), young panicle (7), pistil (8), stamen (9), young embryo (10), SEM picture of rice leaf cuticle (11), cross-sections of stem (12) and leaf (13), and semithin sections of leaf (14 and 15). Red arrows (in 1, 2, 9, 10, 12, 13, 14, and 15) indicate the specific staining sites. Red arrows in 2 and 11 and the blue arrow in 15 point to the epidemic hook hairs above the silica-cork cell lines. (*C*) Responses of *DWA1* to abiotic stress and phytohormone treatments. ABA, abscisic acid; Eth, ethylene; GA, gibberellic acid; IAA, indole-3-acetic acid; JA, jasmonic acid; MV, methyl viologen.

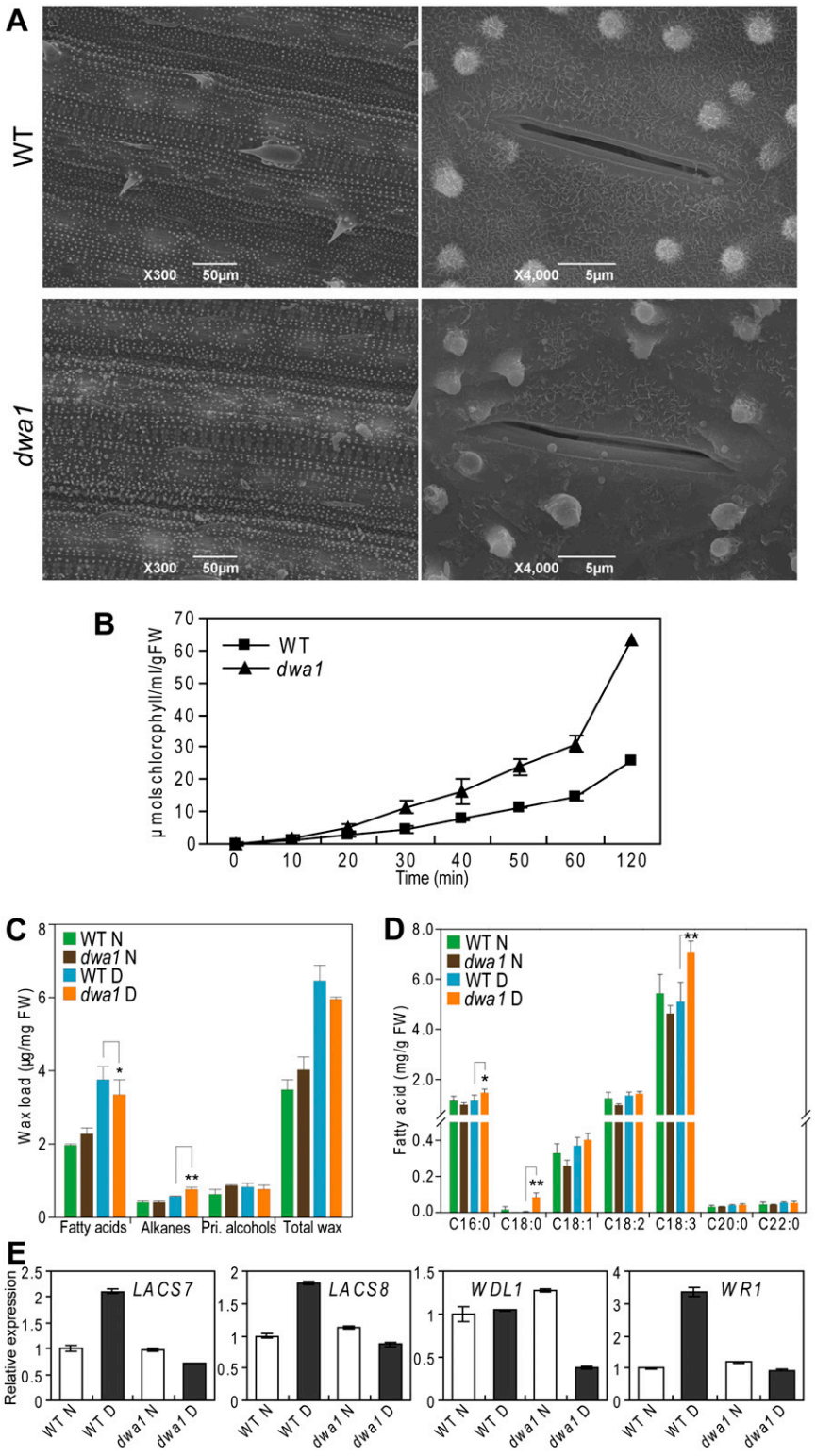

Fig. S4. Alteration of leaf cuticle properties, cuticular wax amount, endogenous FA levels, and wax-related genes in *dwa1* and WT plants under drought stress. D, drought stress; N, normal growth. (*A*) Epicuticular wax and stomatal properties of *dwa1* leaves compared with WT under drought treatment. (*B*) Chlorophyll leaching assays of drought-treated *dwa1* and WT seedlings. Three repeats were averaged. Error bars indicate SE of the mean. (*C*) Cuticular wax amount in *dwa1* leaves compared with WT under both conditions. FW, fresh weight; Pri. alcohols, primary alcohols. Error bars indicate SE of three biological repeats (*t* test: $*P < 0.05$; $**P < 0.01$). (*D*) Endogenous FA level in *dwa1* compared with WT under both conditions. (*E*) Expression level of additional wax-related rice genes in *dwa1* and WT plants under drought stress. Top-second leaves of 4-wk-old WT and mutant plants under both normal and drought conditions were used for quantitative RT-PCR analysis. LACS, long-chain acyl-CoA synthetase; *WDL1*, *wilted dwarf and lethal 1*; *WR1*, *wax synthesis regulatory gene 1*.

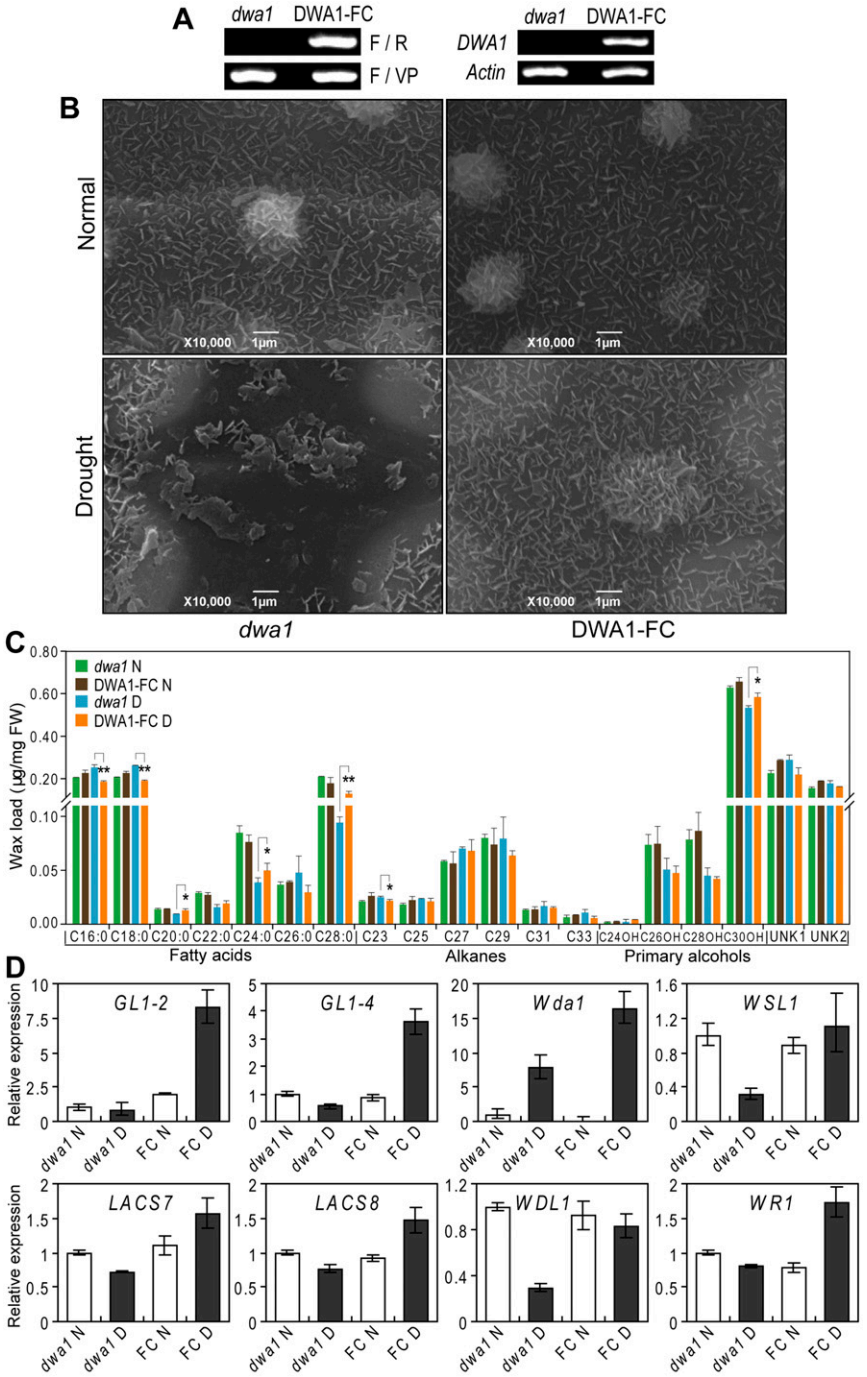

Fig. S5. Leaf cuticular wax pattern, profile, and expression levels of wax-related genes in *dwa1* and DWA1-functional complementation (FC) complemented seedling plants under normal and drought conditions. (*A*) Genotyping the DWA1-FC complemented plants (*Left*) and recovery of *DWA1* transcription in the DWA1-FC plants compared with *dwa1* mutant by RT-PCR analysis (*Right*). VP, vector primer. (*B*) SEM analysis of the restored wax pattern on the leaf cuticle of DWA1-FC seedlings compared with the *dwa1* mutant exposed to drought treatment. (*C*) Cuticular wax composition and loads in DWA1-FC leaves compared with *dwa1* under both normal and drought conditions. Error bars indicate SE of three biological repeats (*t* test: $*P < 0.05$; $**P < 0.01$). UNK, unknown metabolite. (*D*) Expression analysis of wax genes in complemented lines and *dwa1* plants under both normal and drought conditions. *GL*, *GLOSSY*; *Wda1*, *wax-deficient anther1*; *WSL1*, w*ax crystal-sparse leaf1*.

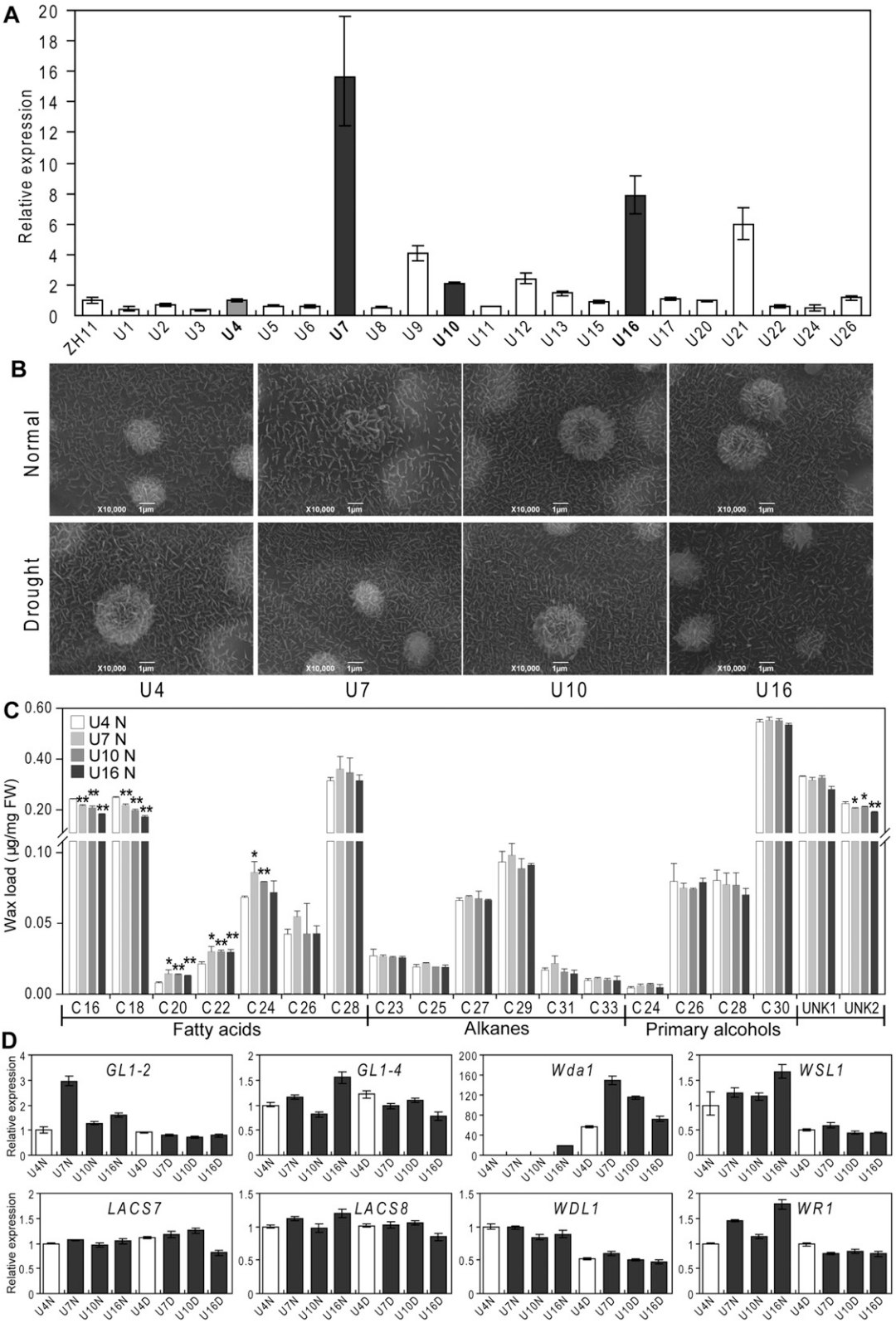

Fig. S6. Leaf cuticular wax pattern, profile, and expression level of wax-related genes in the *DWA1*-overexpression (S131U) plants. (*A*) *DWA1* expression levels in S131U transgenic plants detected by quantitative RT-PCR (qRT-PCR). ZH11 is a WT control; U7, U10, and U16 are three representative independent *DWA1*-overexpression plants; and U4 is one of the transgenic negative controls. (*B*) SEM analysis of wax pattern on the leaf cuticle of *DWA1*-overexpression seedlings under both normal and drought treatments. (*C*) Cuticular wax composition and loads in the leaves of overexpression plants under normal conditions are shown. The data of FAs presented in Fig. 6*A* are included here for comparison. All values are relative to fresh weight and represent the average of three independent experiments. Bars indicate SE of the mean (analyzed using a Student *t* test: $*P < 0.05$; $**P < 0.01$). (*D*) Expression of wax-related genes in *DWA1*-overexpression plants under both conditions analyzed by qRT-PCR.

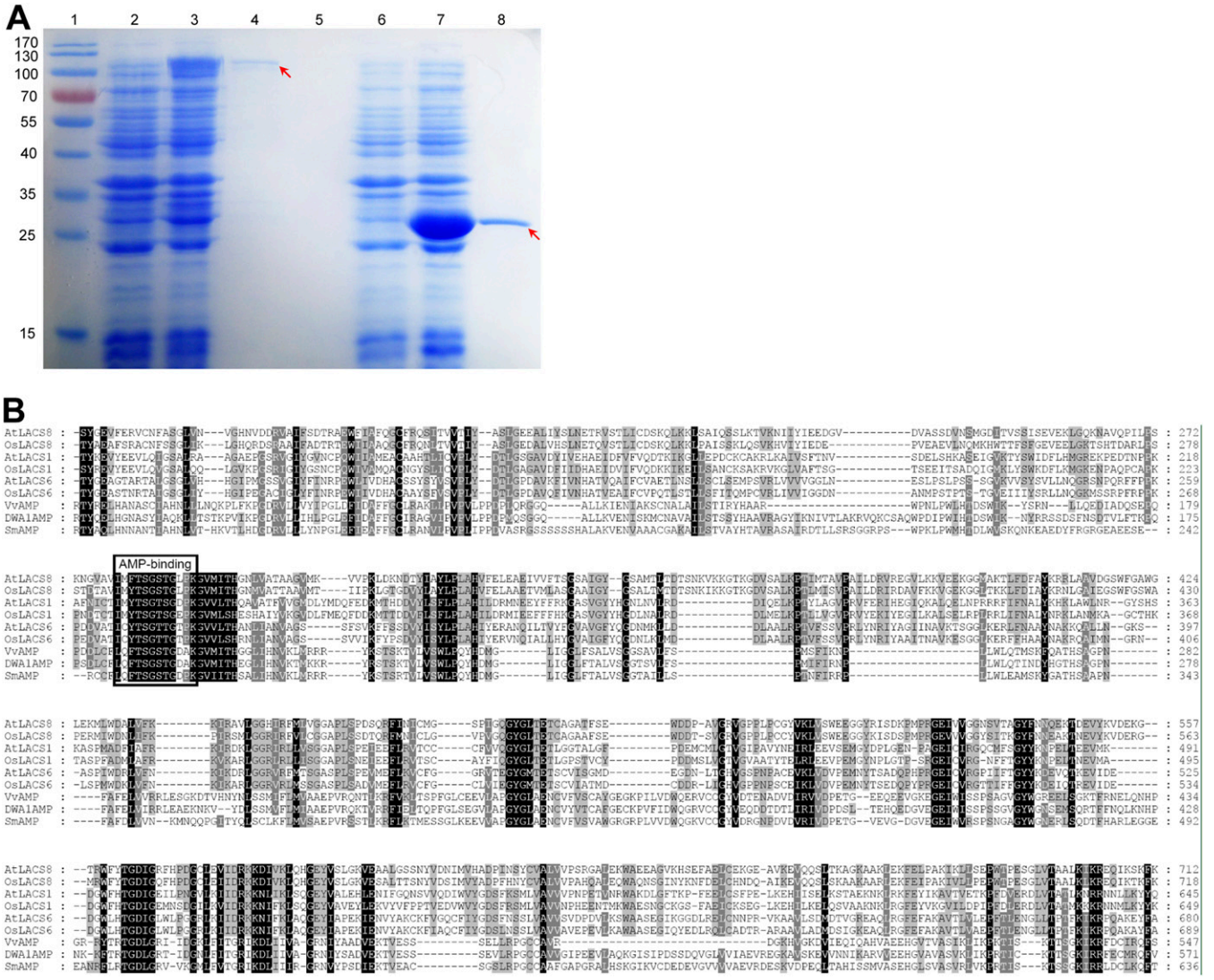

Fig. S7. Expression of DWA1 A domain in *E. coli* and alignment of the A domain with long-chain acyl-CoA synthetases (LACSs). (*A*) Expression of recombinant DWA1 A domain detected by SDS/PAGE. Lane 1, protein ladder (kilodaltons); lane 2, total proteins extracted from cells transformed with pGEX6p-DWA1:AMP-binding domain (AMPbd) without isopropyl β-D-1-thiogalactopyranoside (IPTG) induction; lane 3, total proteins extracted from cells transformed with pGEX6p-DWA1:AMPbd and with IPTG induction; lane 4, purified recombinant DWA1 A domain fused with an N-terminal GST tag; lane 5, no sample added; lane 6, protein extracts from cells transformed with empty vector pGEX-6P-1 without IPTG; lane 7, protein extracts from cells transformed with empty vector pGEX-6P-1 and IPTG induction; lane 8, purified GST protein. Red arrows indicate the purified recombined protein. (*B*) Alignment of the A domain of DWA1 with LACSs from *Arabidopsis* and rice. The rectangular box indicates the core motif for AMP binding.

Table S1. Accessions of DWA1 homologs used for phylogenetic analysis

Protein	Species (vascular plant)	Accession no.	Protein	Species (microorganism)	Accession no.
SmNPSL	*Selaginella moellendorffii*	Gene 108866	AmNPSL	*Aeromicrobium marinum*	ZP_07716202.1
ZmNPSL	*Zea mays*	GRMZM2G165844	AgrNPSL	*Agrobacterium fabrum*	NP_357526.1
SbNPSL	*Sorghum bicolor*	Sb06g019820	MetNPSL	*Methylocystis*	ZP_08072255.1
SiNPSL	*Setaria italica*	SiPROV034918m.g	MitNPSL	*Microbacterium testaceum*	YP_004225560.1
BdNPSL	*Brachypodium distachyon*	Bradi5g13250	MyNPSL	*Mycobacterium gilvum*	YP_004079143.1
MgNPSL	*Mimulus guttatus*	mgv1a021307m.g	NhNPSL	*Nectria hematococca*	XP_003045021.1
AcNPSL	*Aquilegia coerulea*	AcoGoldSmith_v1.018456m.g	ReNPSL	*Rhodococcus equi*	YP_004005363.1
MtNPSL	*Medicago truncatula*	Medtr2g118030	RvNPSL	*Rhodomicrobium vannielii*	YP_004013640.1
CsNPSL	*Cucumis sativus*	Cucsa.153560	SaePLs	*Streptomyces albulus*	BAG68864.1
VvNPSL	*Vitis vinifera*	GSVIVG01025332001	SchNPSL	*Schizophyllum commune*	XP_003033496.1
CpNPSL	*Carica papaya*	evm.TU.supercontig_84.106	StrNPSL	*Streptomyces*	ZP_07280339.1
PpNPSL	*Prunus persica*	ppa027071m.g	TvNPSL	*Trichophyton verrucosum*	XP_003024814.1
PtNPSL	*Populus trichocarpa*	POPTR_0003s22070			
MeNPSL	*Manihot esculenta*	cassava4.1_020910m.g			
RcNPSL	*Ricinus communis*	30169.t000170			

Table S2. Putative stress-related *cis*-elements enriched in the *DWA1* promoter

Name	Sequence	Site, bp	Element/stimulus annotation
ABREOSRAB21	ACGTSSSC	548	ABRE; abscisic acid
CBFHV	RYCGAC	311, 398	CRT/DRE; binding site of CBF/DREB; drought, cold
CRTDREHVCBF2	GTCGAC	398	CRT/DRE; drought, cold
ERELEE4	AWTTCAAA	1,697	Ethylene; senescence
GARE1OSREP1	TAACAGA	858	Gibberellin
GCCCORE	GCCGCC	4, 13, 16, 588, 611	Jasmonic acid
GT1CONSENSUS	GRWAAW	50, 80, 88, 177, 207, 216, 235, 476, 1,328, 1,489	Light
IBOXCORE	GATAA	235, 998, 1,169, 1,249, 1,262, 1,277, 1,975	Light
LTRE1HVBLT49	CCGAAA	1,344	LTRE; low temperature
MYB1AT	WAACCA	192, 889, 1,609, 1,681	rd22BP1; abscisic acid, stress
MYB2CONSENSUSAT	YAACKG	348, 451, 889, 1,047	rd22BP1; abscisic acid, stress
MYBCORE	CNGTTR	348, 451, 670, 859, 889, 1,047, 1,924	Dehydration, stress
MYCATRD22	CACATG	1,312	Dehydration, water stress, abscisic acid
MYCCONSENSUSAT	CANNTG	724, 1047, 1194, 1312, 1,738, 1,752	rd22BP1; abscisic acid, stress, cold
PYRIMIDINEBOXHVEPB1	TTTTTTCC	108, 207	Gibberellic acid, abscisic acid
SORLIP1AT	GCCAC	486, 660, 722, 876	Light

ABRE, abscisic acid responsive element; CBF, C-repeat binding factor; CRT, C-repeat; DRE, dehydration-responsive element; DREB, dehydration-responsive element binding factor; LTRE, low temperature responsive element.

Table S3. Primers used in this study

Gene name	Forward primer	Reverse primer
EL39	CAGTCTTCTCTACATCCGC	CTGTCCTTGTCAGTTGGC
*DWA1*FL	CGGATATCGGAATGCAAGTCAACATGCATGCCTA	CGGATATCCGTCATCGTCCTGCTTACATGCTGTG
*DWA1*Pro	CCCAAGCTTAAAAACTACCGTGACCTTTCCC	CGCGGATCCTGCATGTTGACTTGCATTCC
*DWA1*PE4	CGGAATTCGCCATTGATGGTGAGGGAG	CGGAATTCTGGCTAATGGTGAGGTCAACAG
*DWA1*real	GAAGACTGGGGCTGGGAAA	TGCGTAGATGCTAACGAGGTG
*WDL1*real	GCGACGGCCTGCTTCA	CACAACAATCTTGCTCCCTTCTT
*WSL1*real	GGCGCCAACGACAAGTG	CTTGCCGATCTCGTCTTCCT
*Wda1*real	GCTGCCAAGGAGGGTTATGA	CCCTCCAACGCATGAAGAAT
OsGL1-2	GGCCATCCTGCGTGCA	TCTTATTGAGAGCGGCGAGG
OsGL1-4	TCATCCTGCCGTCGCTG	CGGGAGAGGCTGATCCAGA
*WR1*real	CGGGCAACCTGTCACAGATT	GGCGCCTTGCAGCACTT
*LACS7*real	GTGGACGTGTGAGGCTTATGAC	GGAATTCCATTACATCAGCTGACA
*LACS8*real	GGGAATGCGCTGGTTTTACA	GATCTCGAGGCACCCATCAG
Actin	CTCAACCCCAAGGCTAACAG	ACCTCAGGGCATCGGAAC
Osprofilin1	TGTGGTTTATGTTTGGCATCGTG	ATCTTCATAAAGCAGAACCCACA

PE, protein expression.