Supporting Information

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SI Text

Phenotype Analyses. Seeds were sterilized in 1% (vol/vol) sodium hypochlorite for 1 h and rinsed thoroughly with deionized water. For germination tests, 25 seeds were placed on wet filter paper for 14 days at 25 °C in the light (50 μmol/m^2/s). Seeds were scored as germinated when the coleoptile length was >1 mm. Seeds with 1-mm shoots were transplanted into soil-containing pots and grown in a greenhouse as described in ref. 6. After 14 days, plants were transferred to 7.5-L pots (2 plants per pot) and cultured until seed harvest. Flowering was recorded as the first day when the panicle on the main stem became partly visible. For seed maturation, the date when 50% of the stems of a plant had a visible panicle was recorded and the date when >90% of the filled spikelets were hard and yellow was recorded. The number of days between the 2 events was counted as seed maturation time. For seed set percentage, 3 panicles were randomly selected from each plant. The number of spikelets (filled and empty) was counted, and seed set percentage was calculated (the number of filled spikelets/total number of spikelets × 100).

RNA Extraction and RT-PCR. Total RNA was extracted from embryo-less half seeds by using TRIzol reagent (Invitrogen) as described in the manufacturer's protocol. After isopropanol precipitation, the pellet was resolved in 100 μL of RNase-free water, and the RNA solution was further purified by the RNeasy plant mini kit and treated with RNase-free DNase (Qiagen). cDNA synthesis and RT-PCR analysis were performed by the methods of ref. 6. The level of *Actin1* mRNA was used as a loading control. The number of cycles for logarithmic amplification was carefully optimized in at least 4 technical replications over a range of cycle numbers (e.g., 27, 30, 33, and 36) for each primer pair. Biological replicate samples were analyzed by using the optimal cycle number for each primer pair. Representative results from at least 3 independent biological replicates are presented. Sequences of primer pairs and PCR conditions are listed in Table S1.

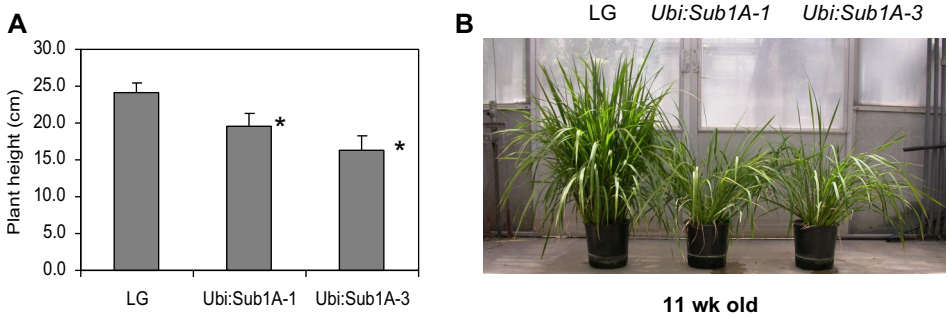

Fig. S1. Ectopic *Sub1A* expression influences vegetative growth. (*A*) Plant height of 14-day-old LG and *Ubi:Sub1A* plants. The data represent mean ± SD ($n = 25$). An asterisk indicates a significant difference between LG and *Ubi:Sub1A* plants ($P < 0.001$). (*B*) Eleven-week-old LG and *Ubi:Sub1A* plants.

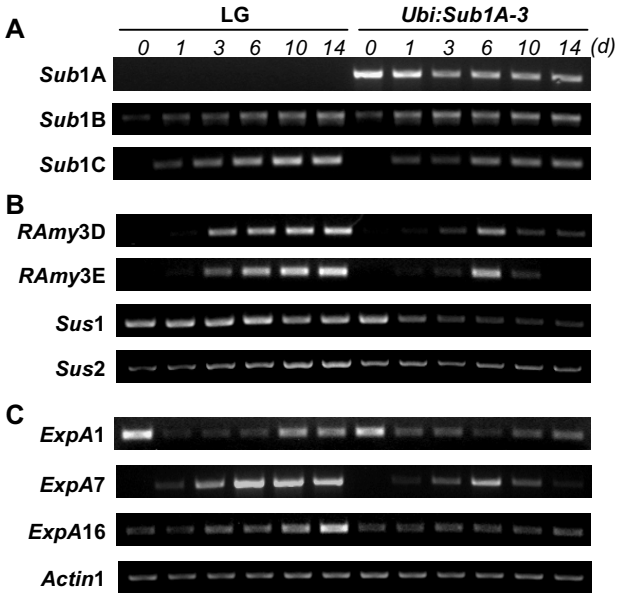

Fig. S2. Accumulation of *Sub1* and other representative gene transcripts in aerial tissue of LG and *Ubi:Sub1A-3* during submergence. Fourteen-day-old (LG) and 21-day-old (*Ubi:Sub1A-3*) plants at a similar developmental age were submerged for up to 14 days, and aerial tissue was collected at specific time points (days 0, 1, 3, 6, 10, and 14). Total RNA was analyzed by semiquantitative RT-PCR by using gene-specific primers. The level of *Actin1* mRNA was used as a loading control. (*A*) *Sub1* genes. (*B*) Genes associated with carbohydrate catabolism; α-amylase (*RAmy*) and sucrose synthase (*Sus*). (*C*) Genes associated with cell elongation; expansin A (*ExpA*).

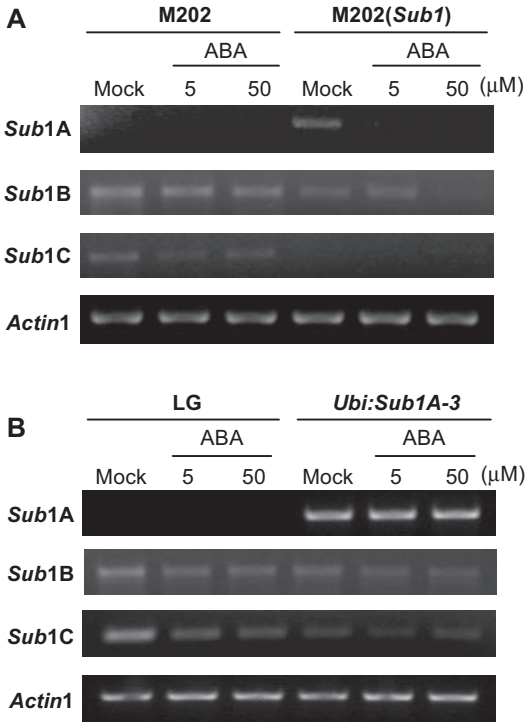

Fig. S3. Accumulation of *Sub1* gene transcripts in response to ABA. (*A*) M202 vs. M202(*Sub1*). (*B*) LG vs. *Ubi:Sub1A-3*. Fourteen-day-old [M202, M202(*Sub1*), and LG] or 21-day-old (*Ubi:Sub1A-3*) plants were treated with mock (0.1% DMSO) or ABA (5 or 50 μM in 0.1% DMSO) solution for 24 h. Total RNA from aerial tissue was analyzed by RT-PCR by using gene-specific primers. The level of *Actin1* mRNA was used as a loading control.

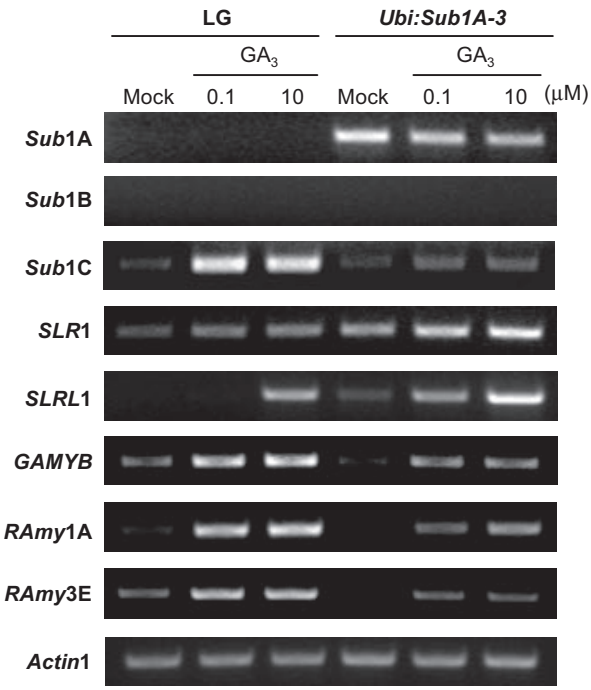

Fig. S4. Transcript accumulation of genes associated with GA signaling in embryo-less half seeds of LG and *Ubi:Sub1A*. Embryo-less half seeds were treated with mock (0.1% DMSO) or GA (0.1 or 10 μM GA_3 in 0.1% DMSO) solution for 24 h. Total RNA from the embryo-less half seeds was analyzed by RT-PCR by using gene-specific primers.

Table S1. Sequences of primers and PCR conditions applied for RT-PCR and quantitative RT-PCR

Gene	Locus name*	Forward primer	Reverse primer	Annealing temperature, °C	Optimal cycles	Expected size, bp
			For RT-PCR			
Actin1	Os03 g50890.1	5′-ACAGGTATTGTGTTGGACTC-3′	5′-GCTTAGCATTCTTGGGTCC-3′	60	25	709
Sub1A	AAAA02037639.1†	5′-AGGTGAAAATGATGCAGG-3′	5′-CTTCCCCTGCATATGATATG-3′	50	28	614
Sub1B	Os09 g11480.1	5′-TTTCCATGTTCCCTTCTGGTG-3′	5′-GCTGCTAATTAACCATTCCCAAAAC-3′	60	38	567
Sub1C	Os09 g11460.1	5′-GTCTGGCTCGGCACCTTC-3′	5′-TGCCGTTTATGTTGTCCTGAATC-3′	60	28	325
SLR1	Os03 g49990.1	5′-ACTTCACCGCAAATCAAGCC-3′	5′-GAGGGAATCGAACATGGTGG-3′	60	28	539
SLRL1	Os01 g45860.1	5′-AAGATCTTCACGGTCATCGAGC-3′	5′-CAAACACACGCTGCTACCATC-3′	60	31	549
GAMYB	Os01 g59660.1	5′-GTTTCCCCAGCACAAAGCAC-3′	5′-CAAACATGACGAATTTCCAACAG-3′	62	23	567
RAmy1A	Os02 g52710.1	5′-CTACGACCATTTCTTCGATTG-3′	5′-GCATAAGCATTAAGCAGTGC-3′	55	24	475
Exp1	Os04 g15840.1	5′-AACGATGGGTGGGGCGTG-3′	5′-TTTACACGCCGACGCCGATG-3′	60	30	727
Exp7	Os03 g60720.1	5′-TCTCCTACCGCCGGGTGC-3′	5′-CCAACCAACATGATGCAGTATCC-3′	60	38	623
Exp16	Os06 g41700.1	5′-TTCCGGAGGGTGAGCTGTGAC-3′	5′-CGTTTGCCATGCTGAGCAGATC-3′	60	30	455
RAmy3D	Os08 g36910.2	5′-TCCCCTGCATCTTCTACGAC-3′	5′-TACTGCATCCTGAACCTGAC-3′	57	27	563
RAmy3E	Os08 g36900.1	5′-TCCCATGCATCTTCTACGAC-3′	5′-ACGAAGCGTACATCGATCG-3′	60	36	431
Sus1	Os03 g28330.1	5′-TTGTGCAGCCCGCTTTCTAC-3′	5′-AAGAGCAGTGCCTAGGAATGC-3′	57	25	578
Sus2	Os06 g09450.1	5′-CGATGAACACAAGTTTGTATTGAAGG-3′	5′-CAAATTCGAATGCAGCTGACG-3′	57	22	819
			For quantitative RT-PCR			
Actin1	Os03 g50890.1	5′-ACAGGTATTGTGTTGGACTCTGG-3′	5′-AGTAACCACGCTCCGTCAGG-3′	60		151
Sub1A	AAAA02037639.1†	5′-CGGCCTCATCACAATCGGAG-3′	5′-ATGTCCATGTCCATATGTCGTCG-3′	60		176
SLR1	Os03 g49990.1	5′-GATCGTCACCGTGGTAGAGC-3′	5′-GAGGGAATCGAACATGGTGG-3′	60		103
SLRL1	Os01 g45860.1	5′-GGCGGCGACAATAACAACAACAGT-3′	5′-TACAAACACACGCTGCTACCATCC-3′	60		125

*TIGR locus name (Nipponbare, *japonica*).
†BGI scaffold name (93–11; *indica*).