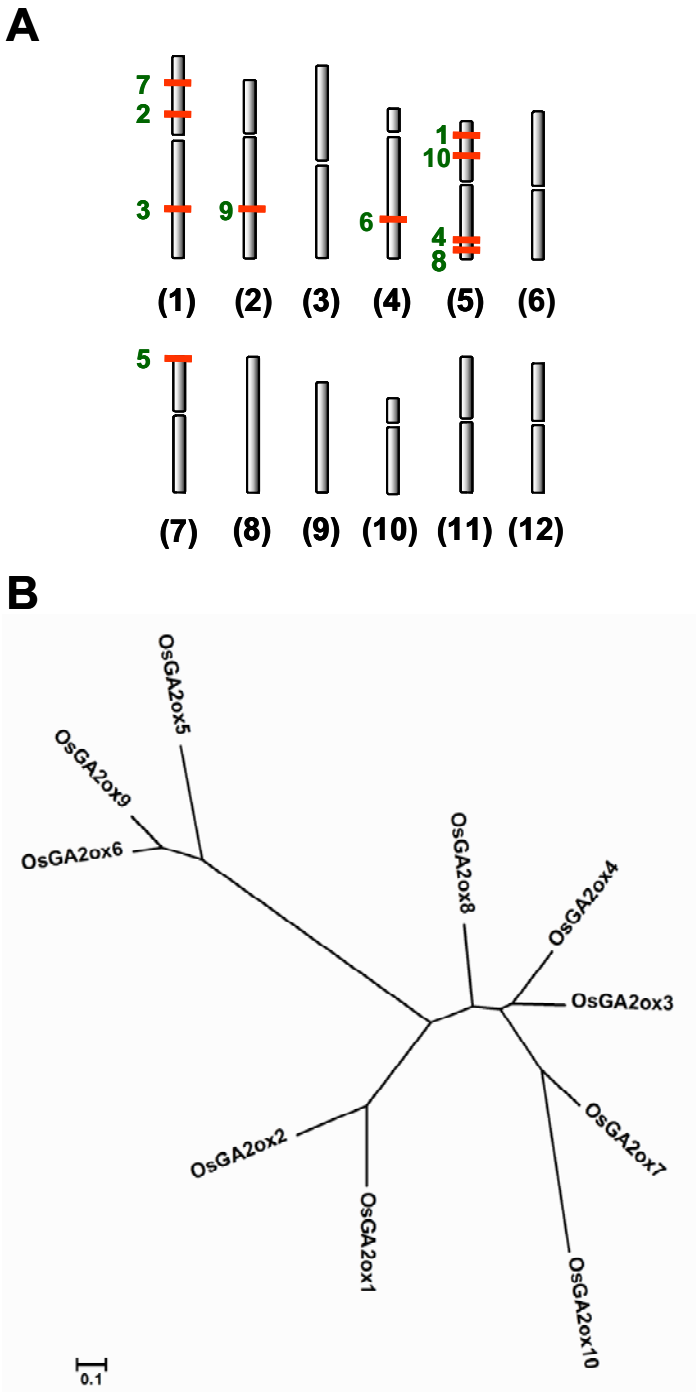
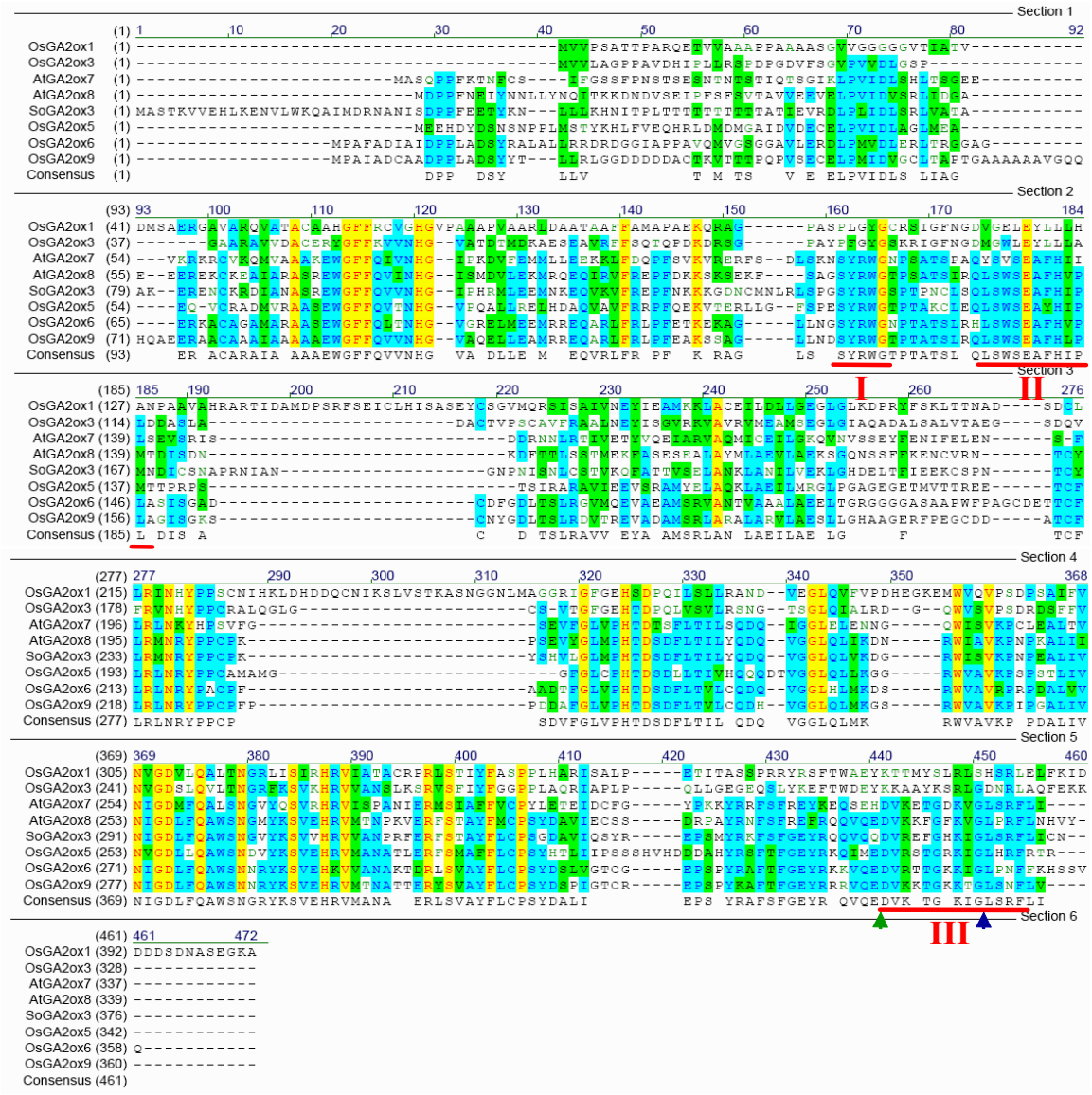


Supplemental Figure 1. The rice GA2ox family.

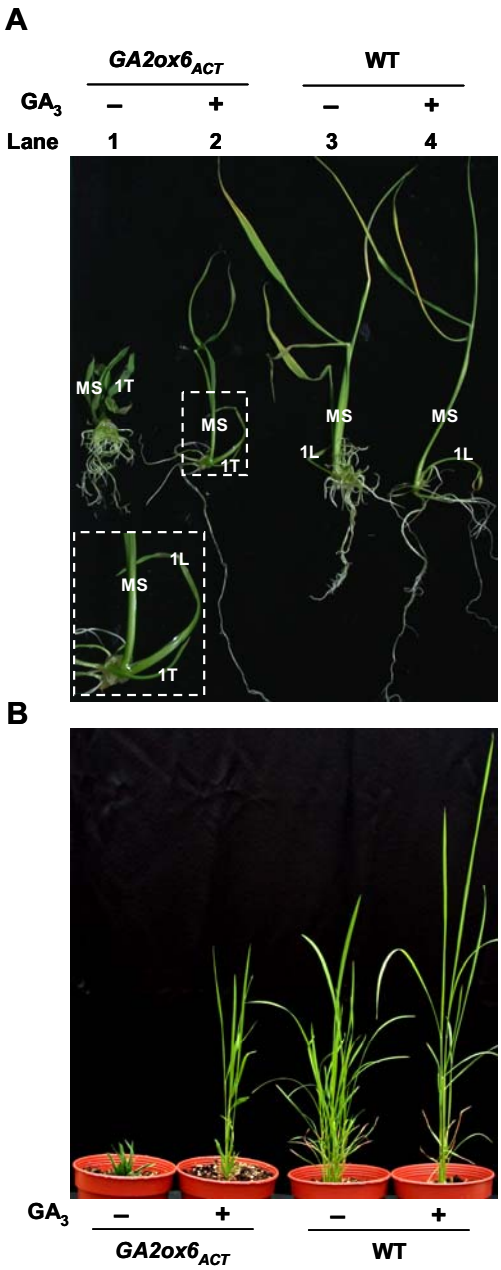
(A) Chromosome locations of *GA2oxs* were determined by the NCBI map viewer program (http://www.ncbi.nlm.nih.gov/mapview/).

(B) Phylogenetic tree based on the comparison of deduced amino acid sequences of rice GA2oxs.

The scale value of 0.1 indicates 0.1 amino acid substitutions per site.



Supplemental Figure 2. C_{20} GA2oxs contain three unique and conserved motifs. Amino acid sequence alignment of rice GA2oxs (OsGA2ox1, OsGA2ox3, OsGA2ox5, OsGA2ox6 and OsGA2ox9), Arabidopsis GA2oxs (AtGA2ox7 and AtGA2ox8) and spinach GA2ox (SoGA2ox3) using the Vector NTI 9.0 software. C_{20} GA2oxs (OsGA2ox5, OsGA2ox6, OsGA2ox9, AtGA2ox7, AtGA2ox8, and SoGA2ox3) contain three highly conserved sequence motifs (underlined with Roman numerals) that are absent in all C_{19} GA2oxs (OsGA2ox1 and OsGA2ox3 as examples for comparison) (Lee and Zeevaart, 2005). The green arrow indicates the position starting from where motifs III in OsGA2ox5 and OsGA2ox6 were deleted (constructs *Ubi:GA2ox5-IIIΔ* and *Ubi:GA2ox6-IIIΔ*, respectively, in Figure 11A). The blue arrow indicates the position where T-DNA was inserted in the *GA2ox5Δ335-341*$_{ACT}$ mutant.



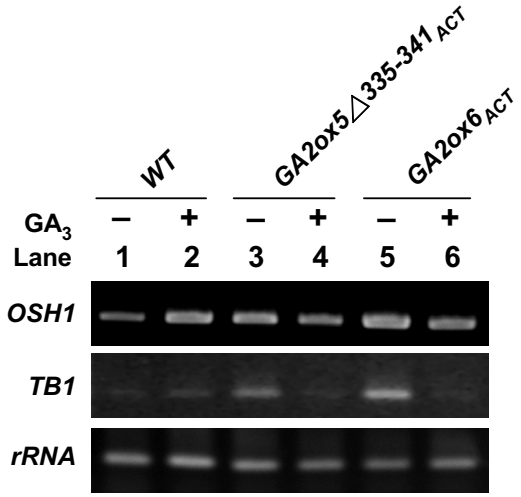
Supplemental Figure 3. GA_3 represses tillering independent of growth stages.

(A) wild type and *GA2ox6$_{ACT}$* mutant seedlings were grown on MS agar medium with or without GA_3 (5 μM) for 15 days. The lower left panel is a higher magnification of the boxed area for the *GA2ox6$_{ACT}$* mutant treated with GA_3 to reveal the main stem and first tiller. MS, main stem; 1T, first tiller; 1L, first true leave.

(B) 1-month-old wild type and *GA2ox6$_{ACT}$* mutant plants were sprayed with 10 μM GA_3 or water only every 7 days for a total of 3 sprays. + and -, presence and absence, respectively.

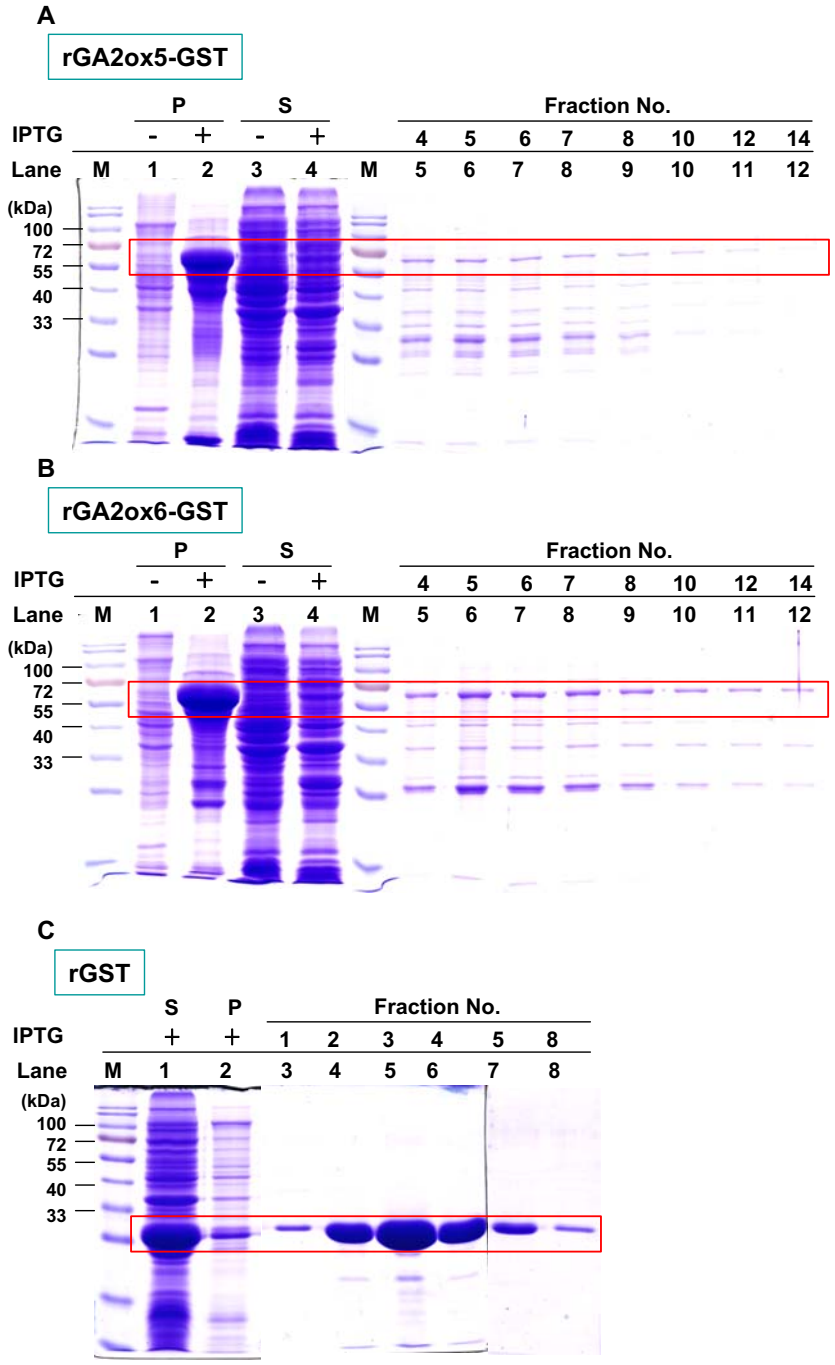
Text for Supplemental Figure 3

To demonstrate that the increase in tiller and adventitious root growth was due to a decrease in endogenous GA levels by overexpression of GA2ox, wild type and *GA2ox6*$_{ACT}$ seedlings were grown in media with or without 5 μM GA_3 after germination. The first tiller of the mutant without GA treatment developed at 9 DAI while that of the GA-treated mutant developed at 16 DAI, indicating that GA_3 delayed tillering (Supplemental Figure 3A). Root system in the GA-treated mutant was also significantly reduced compared to the mutant without GA treatment, suggesting that GA_3 repressed adventitious root development. To further demonstrate that GA inhibits tillering independent of growth stage, 1-month-old wild type and *GA2ox6*$_{ACT}$ plants grown in pot soil were sprayed with 10 μM GA_3 or water only once every 7 days for a total of 3 sprays. Both GA-treated *GA2ox6*$_{ACT}$ mutant and wild-type plants produced 2-3 times fewer tillers than mutant and wild-type plants without GA treatment (Supplemental Figure 3B).



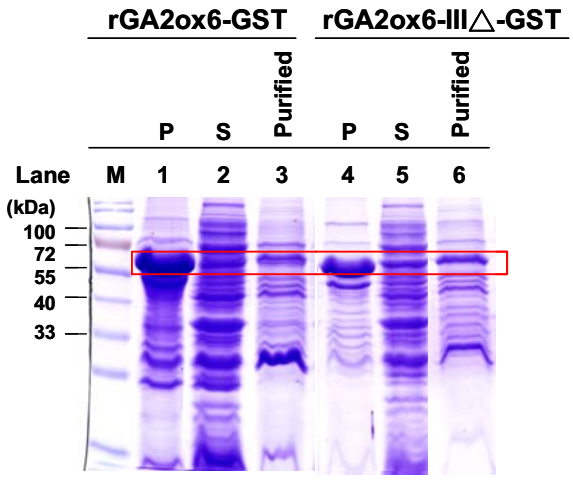
Supplemental Figure 4. GA_3 suppresses *OSH1* and *TB1* expression.

Wild type and $GA2ox6_{ACT}$ and $GA2ox5\Delta335\text{-}341_{ACT}$ mutant seeds were germinated on MS agar medium with (+) or without (-) 5 μM GA_3. Total RNAs were isolated from embryos that containing tiller buds at 12 DAI and analyzed by RT-PCR using primers that specifically amplified rice *OSH1* and *TB1* cDNAs.



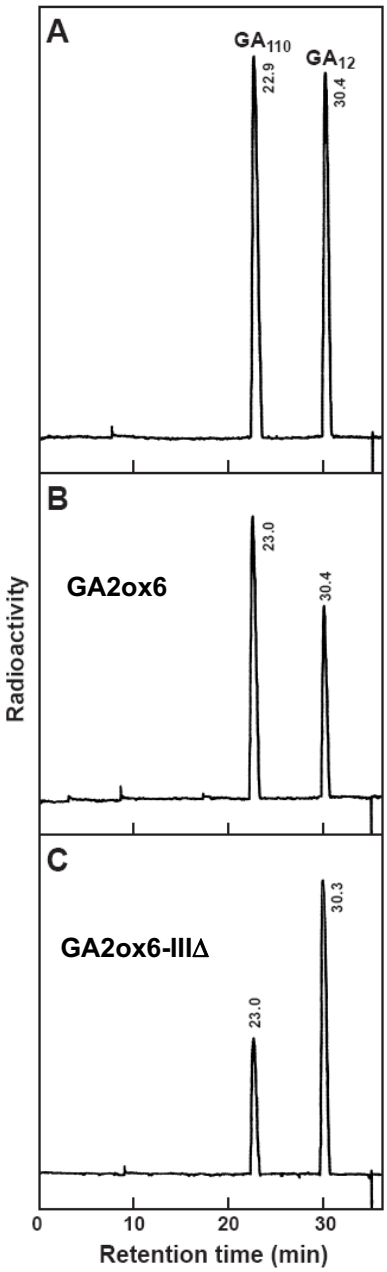
Supplemental Figure 5. Production, purification and SDS-PAGE analyses of GST-fused recombinant (r) GA2ox5 and GA2ox6.

Recombinant proteins were produced in bacteria culture with (+) or without (-) 0.3 μM IPTG. **(A)** rGA2ox5-GST and **(B)** rGA2ox6-GST formed inclusion bodies and mainly present in pellet (P) (lane 2), while **(C)** control rGST was mainly present in supernatant (S) (lane 1). Fractionation of supernatants recovered relatively low amounts of rGA2ox6-GST and rGA2ox5-GST as compared with rGST. Boxes on gels indicate positions of target recombinant proteins. M: Molecular weight marker.



Supplemental Figure 6. Production, purification and SDS-PAGE analyses of GST-fused recombinant (r) GA2ox6 and GA2ox6-III∆.

Recombinant proteins were produced in bacteria culture with 0.3 μM IPTG. rGA2ox6-GST and rGA2ox6-III∆-GST formed inclusion bodies and were largely present in pellet (P) (lanes1 and 4). Recombinant proteins were purified from supernatants (S) (lane2 and 5), pooled (lanes 3 and 6) and assayed for enzyme activity. The box on gel indicates the position of target recombinant proteins. M: Molecular weight marker.



Supplemental Figure 7. Conversion of [^{14}C]GA$_{12}$ to [^{14}C]GA$_{110}$ by recombinant GA2ox6 and GA2ox6-IIIΔ proteins.

(A) Standards of [^{14}C]GA$_{12}$ and [^{14}C]GA$_{110}$.

(B) Conversion of [^{14}C]GA$_{12}$ to [^{14}C]GA$_{110}$ by recombinant GA2ox6 protein.

(C) Conversion of [^{14}C]GA$_{12}$ to [^{14}C]GA$_{110}$ by recombinant GA2ox6-IIIΔ protein.

HPLC analysis was performed with an analytical column (30x0.4 cm) packed with µBondapak C$_{18}$, which was attached to a C$_{18}$ Guard-PAK precolumn. GAs were eluted with a 25-min linear gradient of 30% to 90% methanol in 0.2% aqueous acetic acid at a flow rate of 2.0 ml. min^{-1}. The numbers at the peaks indicate retention times in minutes.

Supplemental Table 1. Putative GA2ox gene family in rice (*Oryza sativa*).

Gene Name	Chromosome	BAC No.	Site[a]	Locus[b]	Accession no. of cDNA	PI value	Amino acid
GA2ox1	5	OSJNBa0017J22	8748 ~ 34623	LOC_Os05g06670	AK120967	6.52	403
GA2ox2	1	B1140D12	9818 ~ 19799	LOC_Os01g22910		6.64	370
GA2ox3	1	OJ1414_E05	66551 ~ 68322	LOC_Os01g55240	AK101713	6.26	327
GA2ox4	5	P0022D06	49345 ~ 50496	LOC_Os05g43880	AK107211	6.39	354
GA2ox5	7	P0446F04	52078 ~ 53103	LOC_Os07g01340	AK106859	5.88	341
GA2ox6	4	OSJNBa0019D11	138689 ~ 141990	LOC_Os04g44150	AK107142	7.18	358
GA2ox7	1	P0466B10	35930 ~ 39272	LOC_Os01g11150	AK108802	6.67	335
GA2ox8	5	OJ1115_B06	55485 ~ 57032	LOC_Os05g48700	AK101758	6.03	353
GA2ox9	2	B1469H02	122528 ~ 120151	LOC_Os02g41954.1	Ak059045	5.58	359
				LOC_Os02g41954.2	AK108598	5.04	299
GA2ox10	5	OSJNBb0016G07	9180 ~ 13210	LOC_Os05g11810.1		9.37	378
				LOC_Os05g11810.2		9.33	271

[a] The critical site of BAC clone. [b] Locus was identified by the TIGR Rice Pseudomolecules and Genome Annotation 5.0 (http://www.tigr.org/ tdb/e2k1/osa1/).

Supplemental Table 2. Comparison of deduced amino acids among rice GA2oxs.

	GA2ox5	GA2ox6	GA2ox9	GA2ox1	GA2ox2	GA2ox10	GA2ox7	GA2ox3	GA2ox4	GA2ox8
GA2ox5	100	62	63	38	43	22	47	50	47	45
GA2ox6		100	76	36	41	20	44	46	44	44
GA2ox9			100	34	39	19	43	45	42	42
GA2ox1				100	66	24	49	50	49	51
GA2ox2					100	32	56	57	56	57
GA2ox10						100	41	38	35	35
GA2ox7							100	70	63	64
GA2ox3								100	73	70
GA2ox4									100	67
GA2ox8										100

Numbers denote percentage identity between predicted proteins. Thick lines separate C_{20} GA2oxs from C_{19} GA2oxs.

Supplemental Table 3. Gene names and accession numbers of 19 GA2oxs from different plant species.

Name	Species	Accession Number
AtGA2ox1	*Arabidopsis thaliana*	AJ132435
AtGA2ox2	*Arabidopsis thaliana*	AJ132436
AtGA2ox3	*Arabidopsis thaliana*	AJ132437
AtGA2ox4	*Arabidopsis thaliana*	AY859740
AtGA2ox6	*Arabidopsis thaliana*	AY859741
AtGA2ox7	*Arabidopsis thaliana*	NM103976
AtGA2ox8	*Arabidopsis thaliana*	NM118239
CmGA2ox	*Cucurbita maxima*	AJ302041
LsGA2ox1	*Lactuca sativa*	AB031206
NtGA2ox1	*Nicotiana sylvestris*	AB125232
NtGA2ox3	*Nicotiana sylvestris*	EF471117
NtGA2ox5	*Nicotiana sylvestris*	EF471118
PcGA2ox1	*Phaseolus coccineus*	AJ132438
Poplar GA2ox1	*Populus alba x P. tremuloides*	AY392094
PsGA2ox1	*Pisum sativum*	AF056935
PsGA2ox2	*Pisum sativum*	AF100954
SoGA2ox1	*Spinacia oleracea*	AF506281
SoGA2ox2	*Spinacia oleracea*	AF506282
SoGA2ox3	*Spinacia oleracea*	AY935713

Supplemental Table 4. Primers used for T-DNA flanking sequence, PCR and RT-PCR analyses and plasmid constructions.

Primers	Sequence	Gene
PCR (Confirmation of T-DNA insertion and genotyping)		
GA2ox5-5'	5'- ATGGAGGAGCACGACTACGACT -3'	*OsGA2ox5△335-341*$_{ACT}$ genotyping
GA2ox5-R2	5' TCCTCCATGATCTGCTTCCTGTA -3'	
GA2ox6-5'	5'- AGATACTCACTCCGTTTCATGTT -3'	*OsGA2ox6*$_{ACT}$ genotyping
GA2ox6-3'	5'- GTAGTGCGGTGAAACAGGATGCC -3'	
GA2ox9-5'	5'- TGCTCCGGACGCCACAATCTA -3'	*OsGA2ox9*$_{ACT}$ genotyping
GA2ox9-3'	5'- CGAGATGATACTTTGACCAACAAT -3'	
RB	5'- AACTCATGGCGATCTCTTACC-3'	T-DNA right border
RT-PCR- analysis of gene expression		
GA2ox1-F	5'- CGAGCAAACGATGTGGAAGGGCTACAGG -3'	*OsGA2ox1* (332 bp)
GA2ox1-R	5'- TGGCTCAGGCGGAGTGAGTACATTGTCG -3'	
GA2ox2-F	5'- CCCCACATCCCTGACAAGGCTC -3'	*OsGA2ox2* (592 bp)
GA2ox2-R	5'- CTATTCATGGTCGTCATCGTCC -3'	
GA2ox3-F	5'- TGAGCGCGCTGGTGACGGCGGA -3'	*OsGA2ox3* (451 bp)
GA2ox3-R	5'- CTTGATTTGTAGGCAGCCTTC -3'-	
GA2ox4-F	5'- TCGGTGGAGGATAACTTCGGC -3'	*OsGA2ox4* (999 bp)
GA2ox4-R	5'- TGGGTTAGCGACAGGTGGTGG -3'	
GA2ox5-F	5'- ATGGAGGAGCACGACTACGACT -3'	*OsGA2ox5* (974 bp)
GA2ox5-R	5' TCCTCCATGATCTGCTTCCTGTA -3'	
GA2ox6-F	5'- GACGACGTGCTTCCTGCGGCTCAA-3'	*OsGA2ox6* (389 bp)
GA2ox6-R	5'- CTTCCTGCACCTTCTTCCTGTA-3'	
GA2ox7-F	5'- ACGGGAGCTTCTACGCGAGT -3'	*OsGA2ox7* (594 bp)
GA2ox7-R	5'- TCAAATCTGCAGAGCCTGTCGTC -3'	
GA2ox8-F	5'- GTGCTGCGGCGGATGGTGGTGG -3'	*OsGA2ox8* (555 bp)
GA2ox8-R	5'- CTTCGTCGCGGCCTCATCGTTGG -3'	
GA2ox9-F	5'- ATGTCGAGGCTGGCCAGGG -3'	*OsGA2ox9* (533 bp)
GA2ox9-R	5'- CATACGAGGAAATTACTGAGGC -3'	
GA2ox10-F	5'- ATGAGACAGCTCCGCCGTCTCTGG -3'	*OsGA2ox10* (414 bp)
GA2ox10-R	5'- TTACGTCGTTGTGTTCGATCGTC -3'	
GA2ox11-F	5'- CTCCGATCCAACGACACCTCT -3'	*OsGA2ox11* (501 bp)
GA2ox11-R	5'- AGCCAGCGCCTCGTCCTGAT -3'	
GA3ox2-F	5'- TCTCCAAGCTCATGTGGTCCGAGGGCTA -3'	*OsGA3ox2* (346 bp)
GA3ox2-R	5'- TGGAGCACGAAGGTGAAGAAGCCCGAGT -3'	
18S-F	5'- CCTCGTGCCCCTATCAACTT-3'	*18S rRNA* (201 bp)
18S-R	5'- GACACTAAAGCGCCCGGTAT-3'	
OSH1-F	5'-GAGATTGATGCACATGGTGTG-3'	*OSH1* (737 bp)
OSH1-R	5'-ATTAGCAGCAGCAAGAGTAGC-3'	RT-PCR
OsTB1-F	5'-AAGTTCTTCGCGCTCCAGGA-3'	*OsTB1* (508 bp)
OsTB1-R	5'-GATTGGTGGACGATGAGTGG-3'	RT-PCR
OSH1-F	5'-GAGATTGATGCACATGGTGTG-3'	*OSH1* (183 bp)
OSH1-R-2n	5'-CGAGGGGTAAGGCCATTTGTA-3'	Quantitative RT-PCR
OsTB1-F-2n	5'-CAAGGAGAAGAACCGGATGCG-3'	*OsTB1* (140 bp)
OsTB1-nR	5'-GATTGGTGGACGATGAGTGG-3'	Quantitative RT-PCR

Supplemental Table 4. Primers used for T-DNA flanking sequence, PCR and RT-PCR analyses and plasmid constructions (continued).

Primers	Sequence	Gene
RT-PCR- cDNA amplification for cloning		
GA2ox5-full-F	5'- AGC**GGATCC**ATGGAGGAGCACGACTACG -3'	*OsGA2ox5* full length
GA2ox5-full-R	5'- AAT**GGATCCC**TATCGGGTTCGAAAGCGG -3'	(for cloning)
GA2ox6-full-F	5'- TT**GGATCC**ATGCCGGCCTTCGC-3'	*OsGA2ox6* full length
GA2ox6-full-R	5'- CG**GGATCC**TTATTGTACTGAAGA-3'	(for cloning)
GA2ox5-III-D-R	5'- TC**GGATCC**CTACTCCATGATCTGCTTCCTG -3'	cloning of *Ubi:OsGA2ox5-III△*
GA2ox6-III-D-R	5'- TTT**GGATCC**TTATTCCTGCACCTTCTTCCT -3'	cloning of *Ubi:OsGA2ox6-III△*