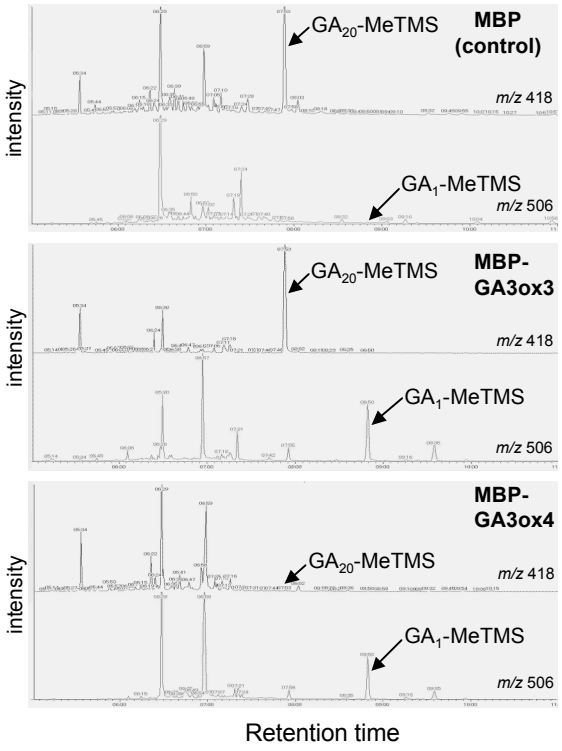
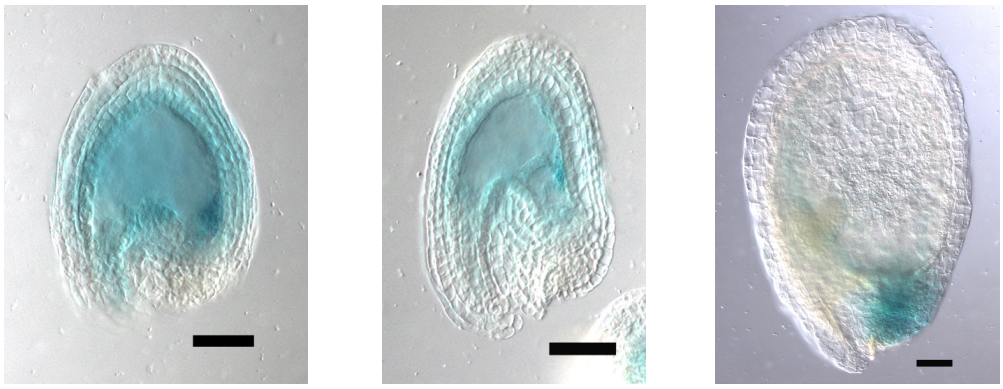
Supplemental data. Hu et al. (2008). Potential sites of bioactive gibberellin production during reproductive growth in *Arabidopsis*.



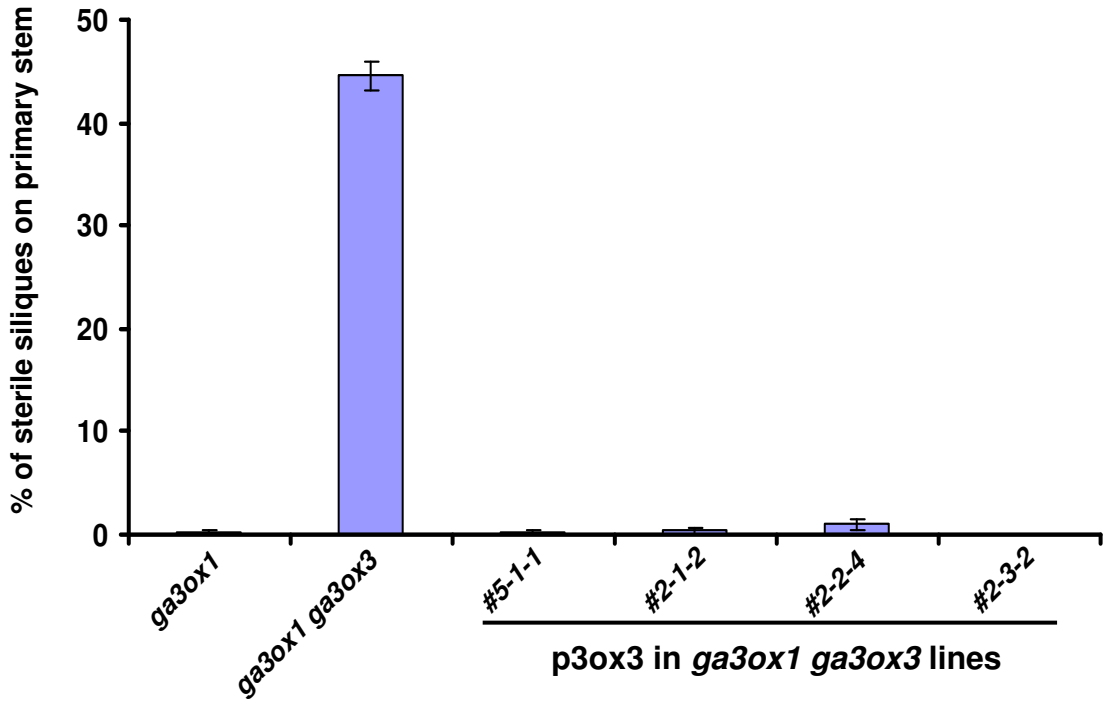
Supplemental Figure 1. WT GA3ox3 and GA3ox4 proteins convert GA_{20} to GA_1 in in vitro enzyme assays .

GC-selected ion monitoring showing the conversion of GA_{20} to GA_1 by maltose binding protein(MBP)-GA3ox3 and MBP-GA3ox4 fusion protein (the latter panels, respectively). Cell lysates containing MBP were used as a negative control (the first panel). GA_{20} and GA_1 were identified as GA_{20}- methyl ester-trimethylsilyl ether (GA_{20}-MeTMS) and GA_1- methyl ester-trimethylsilyl ether (GA_1-MeTMS), respectively, after derivatization.

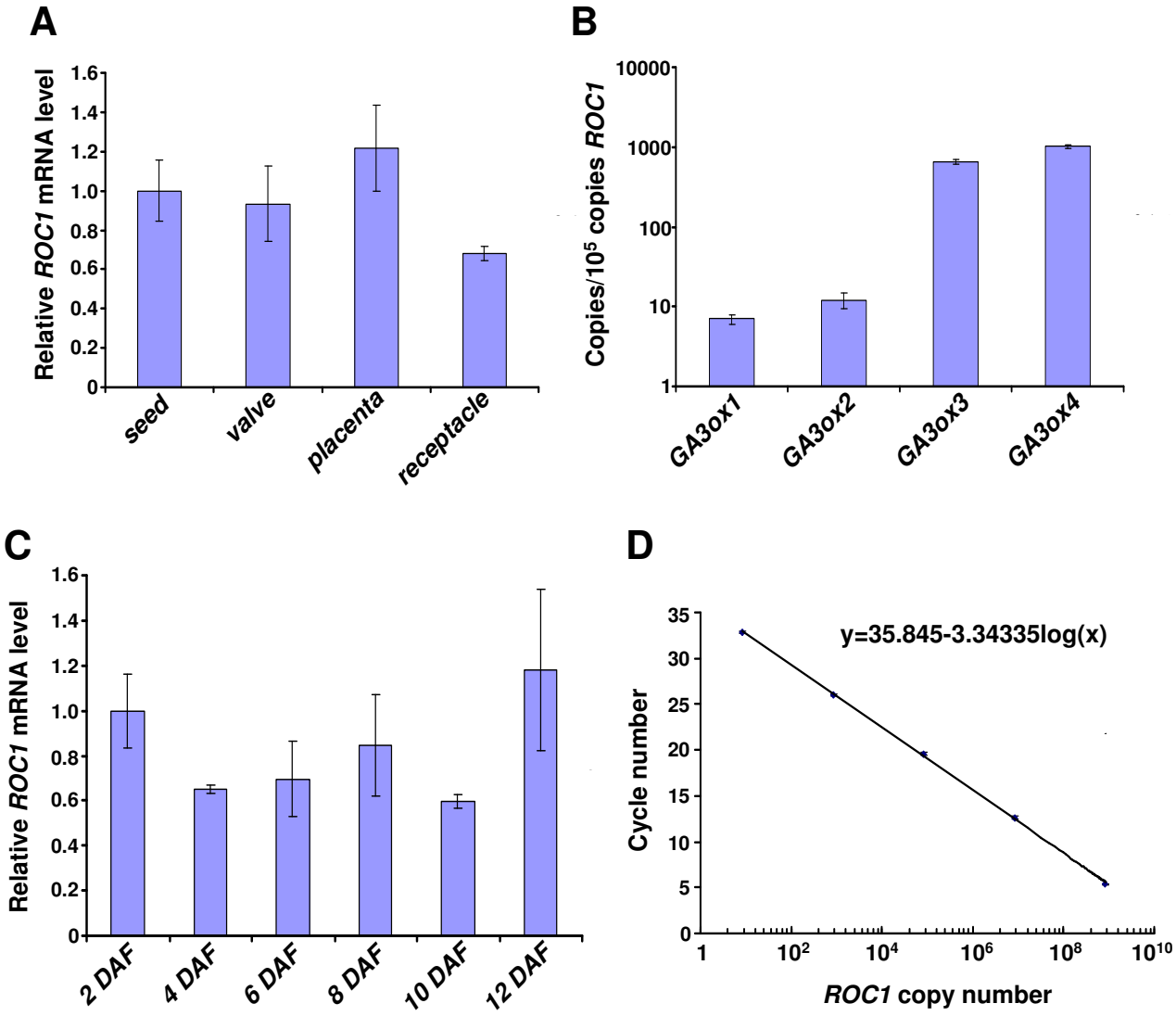


Supplemental Figure 2. *GA3ox4-GUS* expression in embryos resulted from crosses between *GA3ox4-GUS* plants (pollen donors) and WT plants.

DAP: Days after pollination. Bar: 50 μM.

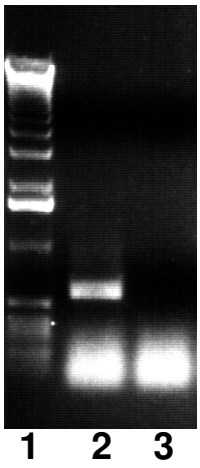


Supplemental Figure 3. The p3ox3/*ga3ox1 ga3ox3* transformants rescued the sterile phenotype of *ga3ox1ga3ox3*.

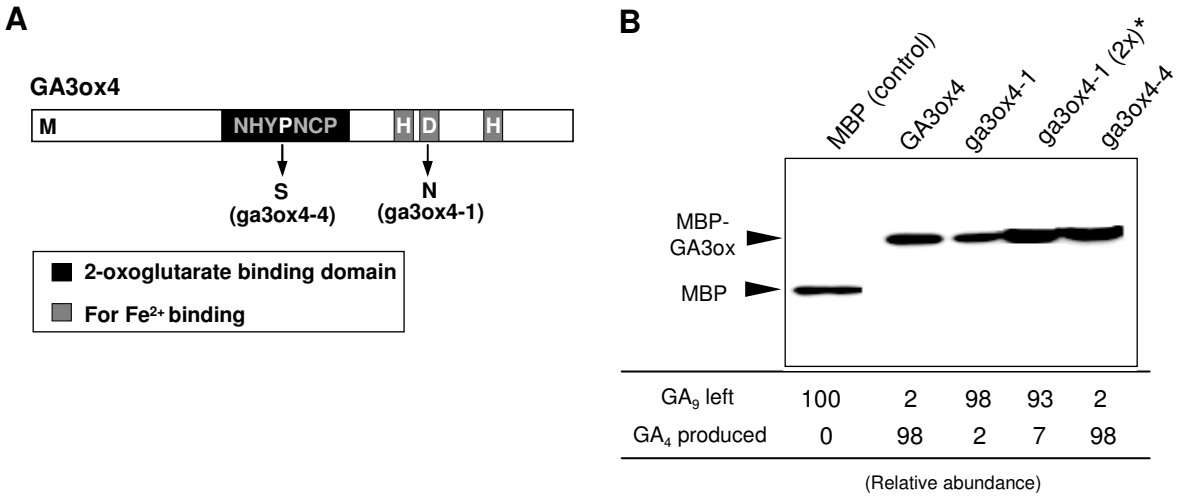


Supplemental Figure 4. Expression of *ROC1* and *GA3ox* genes as determined by qRT-PCR.

(A) Relative expression of *ROC1* in different parts of 6-8 DAF siliques, normalized by 18S rRNA levels. (B) Semi-log graph of *GA3ox* transcript levels in seeds of 6-8 DAF siliques. (C) Relative expression of *ROC1* in different silique stages, normalized by 18S rRNA levels. Means ± S.E. of three technical replicates are shown in A-C. (D) Standard curve for *ROC1* quantification in real-time PCR.



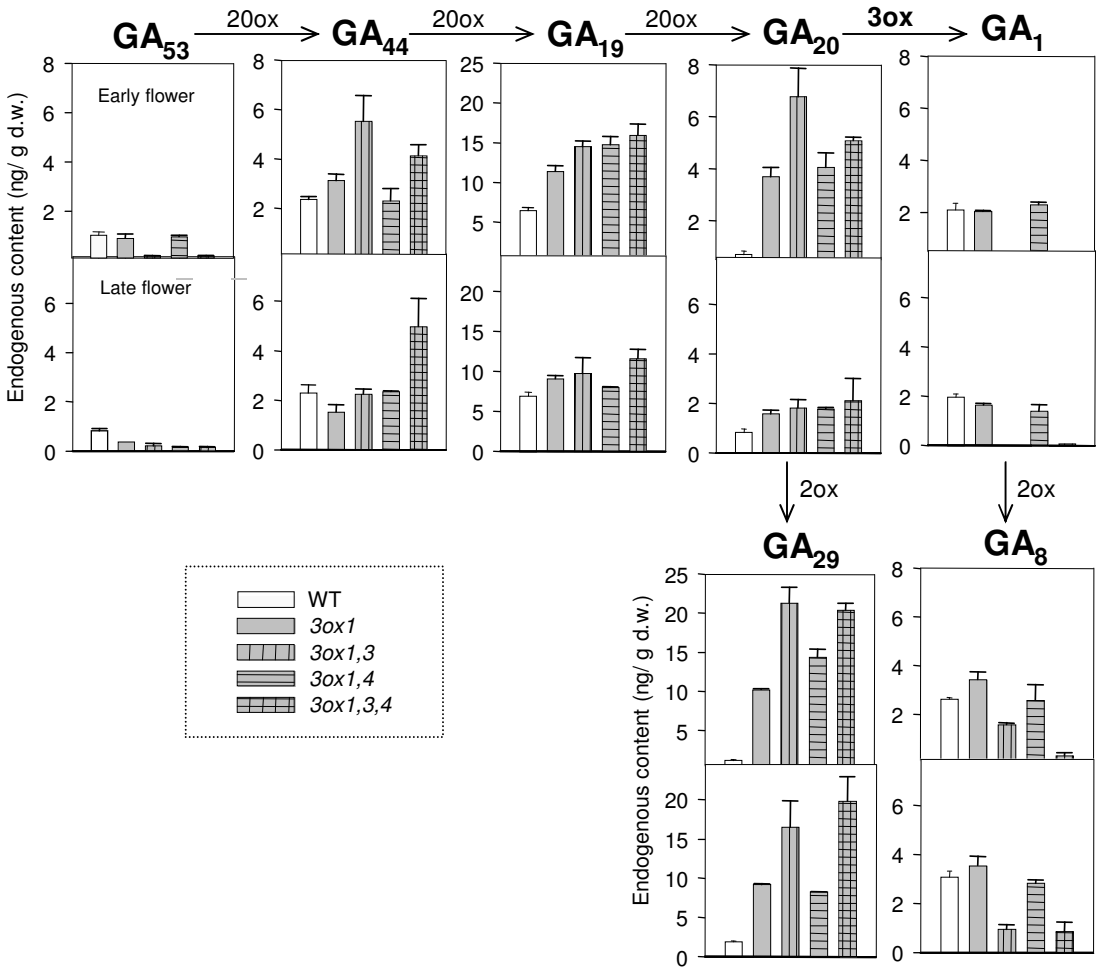
Supplemental Figure 5. The *ga3ox3* mutant has no WT *GA3ox3* transcript.
RT-PCR was performed using RNA isolated from siliques to detect WT *GA3ox3* transcripts. 1. 1 kb DNA ladder. 2. WT silique RNA was used for RT-PCR. 3. *ga3ox3* silique RNA was used for RT-PCR.



Supplemental Figure 6. In vitro enzyme assays of GA3ox4 and mutant proteins ga3ox4-1 and ga3ox4-4.

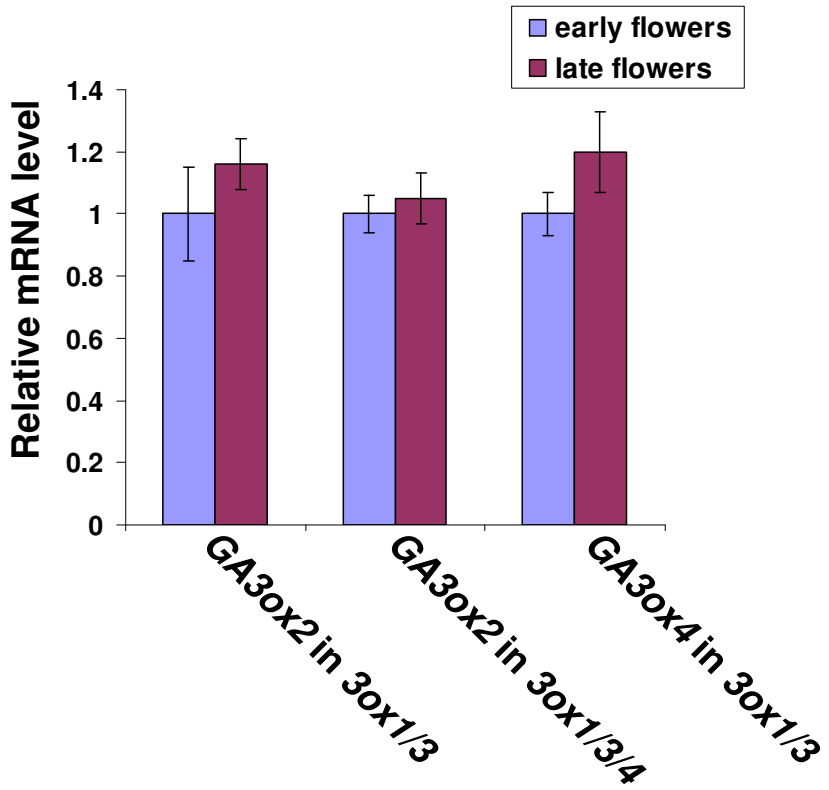
(A) Positions of amino acid substitutions in the ga3ox4-1 and ga3ox4-4 mutant proteins. Both *ga3ox4-1* and *ga3ox4-4* mutants have a point mutation in the second exon resulting in an amino acid substitution within highly conserved functional domains of 2-oxoglutarate dependent dioxygenases (Xu et al., 1995; Hedden and Phillips, 2000). *ga3ox4-1* has a G-to-A mutation that resulted in an Asp-to-Asn substitution in the protein, which is likely to be inactive because this Asp residue is required for Fe^{2+} binding. *ga3ox4-4* has a nucleotide change from a C-to-T resulting in a Pro-to-Ser amino acid substitution within the 2-oxoglutarate binding domain. The highly conserved functional domains for 2-oxoglutarate dependent dioxygenases (including GA3ox) are depicted.

(B) Immunoblot analysis to detect MBP (control) or MBP-GA3ox fusion proteins in soluble *E. coli* extracts using an anti-MBP antibody. The amount of GA_9 left after the reaction and the amount of GA_4 produced from GA_9 were quantified using deuterium-labeled GAs as internal standards and the relative levels (the sum of the substrate and the product was set to 100) are shown in a table below the blot. The * indicates a sample that contained double the amount of *ga3ox4-1* extract, as the amount of MBP-ga3ox4-1 detected from a similar volume of soluble *E. coli* extracts was smaller than that of MBP-GA3ox4. In vitro assays showed that the *ga3ox4-1* mutation almost completely abolished GA3ox enzymatic activity, as only very little GA_4 was made from GA_9. This result suggested that biochemically, *ga3ox4-1* is a near-null mutant. Surprisingly, the *ga3ox4-4* mutant protein could convert most of the GA_9 into GA_4, indicating that the enzyme activity is not dramatically affected by this mutation.



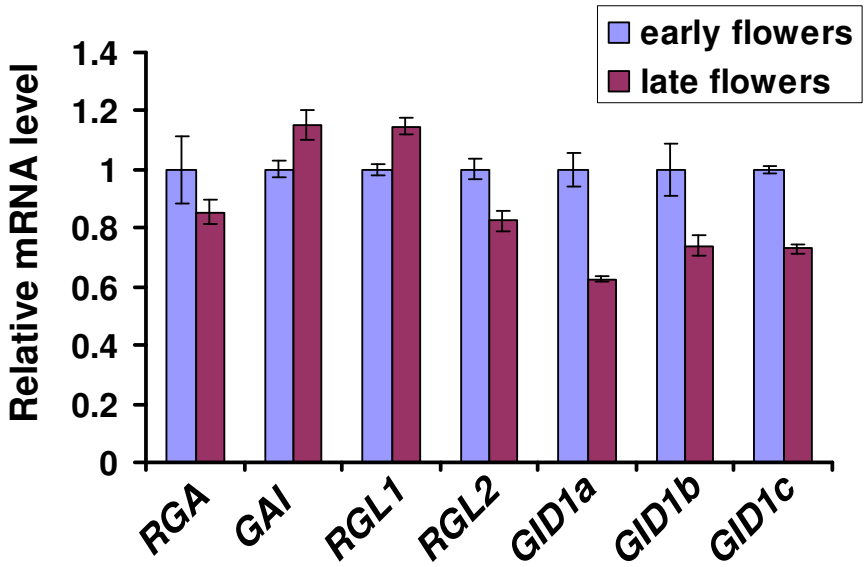
Supplemental Figure 7. Levels of endogenous 13-OH GAs in flowers of WT and category II *ga3ox* mutant plants.

GA levels in early (upper graphs) and late (lower graphs) flowers are presented. Note that the *y*-axis scale for GA_{19} and GA_{29} is different from that for other GAs. Early flowers: flower cluster from the primary inflorescence at anthesis of the first flower. Late flowers: flower cluster from the primary inflorescence after 20-25 flowers already opened. Means ± S.E. of three technical replicates are shown.



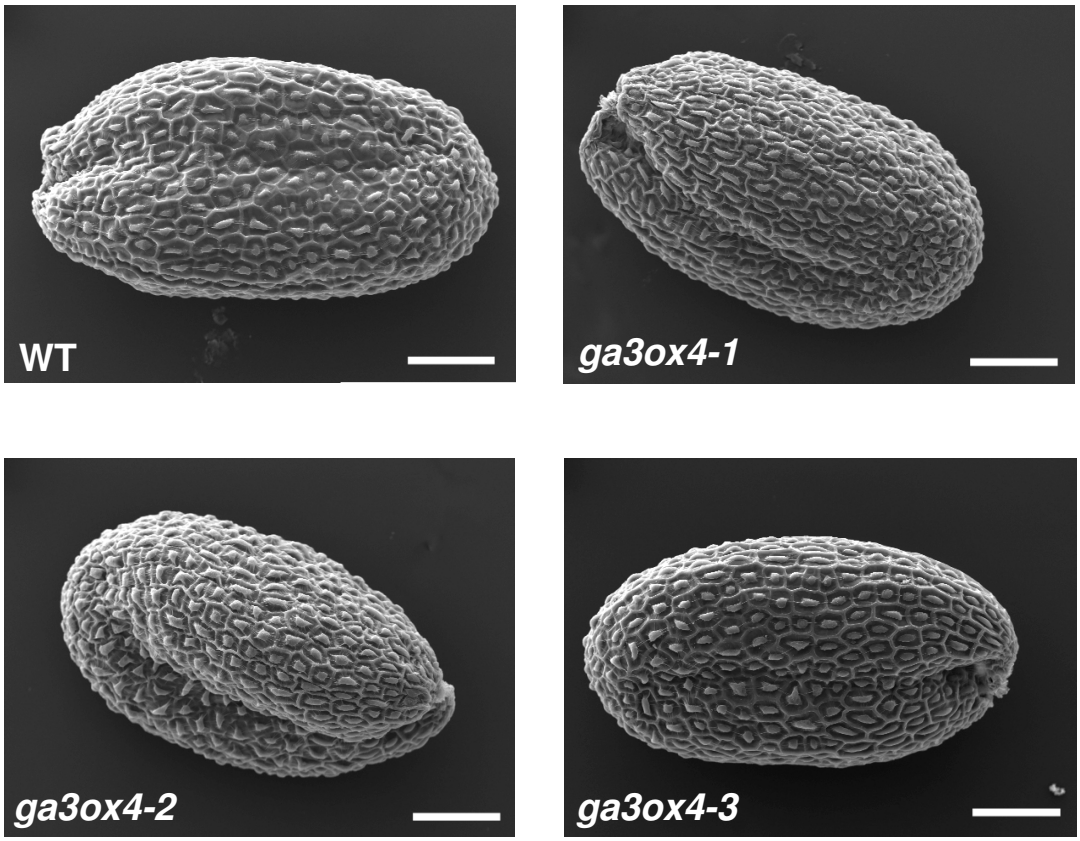
Supplemental Figure 8. *GA3ox2* and *GA3ox4* expression in flowers of *ga3ox* mutants.

Real-time RT-PCR was performed to compare the relative expression levels of *GA3ox2* and *GA3ox4* in early and late flowers of *ga3ox1 3ox3* and *ga3ox1 3ox3 3ox4*. *ROC1* was used as standard. Early flowers and late flowers are defined as in Supplemental Figure 3. Means ± S.E. of three technical replicates are shown. Similar results were obtained in a second test using a different set of tissues.



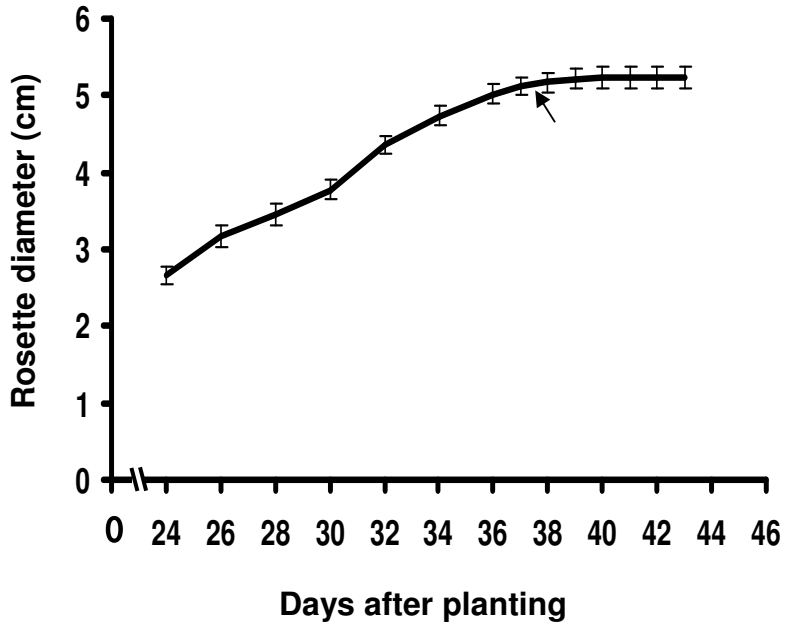
Supplemental Figure 9. Transcript levels of *DELLA* and *GID1* genes in *ga3ox1 ga3ox3 ga3ox4* flowers.

Relative mRNA levels of *DELLA* and *GID1* genes in early and late flowers of *ga3ox1 ga3ox3 ga3ox4* were determined by real-time RT-PCR, using *ROC1* as a standard. PCR primers for *DELLAs* and *GID1s* are described previously (Tyler et al., 2004; Griffiths et al., 2006). Early flowers and late flowers are defined as in Supplemental Figure 3. Means ± S.E. of three technical replicates are shown. An independent test showed similar results.



Supplemental Figure 10. Scanning electron microscopy (SEM) of WT and *ga3ox4* mutant seeds.

Bar: 100 μm.



Supplemental Figure 11. Rosette diameters of *ga3ox1 ga3ox3* after flower initiation.

Arrow: average time when 1st flower opened (37.5 ± 0.3 days).

Silique number	Carpel/Silique stage in Figure 4T	Embryo developmental stage	Silique or flower phenotype	Flower stage according to Bowman (1994)	Silique age according to Bowman (1994)
	carpel		Petals level with long stamens	12	
	anthesis		Bud opens; anther dehiscence; anthesis	13	– 6 HAF
1	0 HAF		Long anthers extend above stigma; fertilization	14	0 HAF
2	10 HAF		Petals perpendicular to carpel	mid-14	
3	20 HAF		Stigma heavily pollinated	end of 14	1 DAF
4	30 HAF		Stigma extends above long anthers	15	
5	40 HAF		Stigma extends above long anthers	15	
6	50 HAF		Petals and sepals withering	15-16	2 DAF
7	60 HAF		All organs fall from siliques	start of 17	2.5 DAF
8	3 DAF			17	
10-11	4 DAF	globular		17	
13-14	5 DAF	heart		17	
15-16	6 DAF	torpedo		17	
17-18	7 DAF	walking stick		17	
20-21	8 DAF	walking stick		17	
25-26	10 DAF	upturned U		17	10.5 DAF
30-31	12 DAF	mature	Siliques turn yellow	18	12 DAF

Supplemental Table 1. List of flower and silique developmental stages.
HAF: hour after flowering.
DAF: day after flowering.

	Flower time (days)	Rosette diameter (cm)	Final height (cm)	% of fertile siliques	Seed per fertile silique
3ox1	22.6 ± 0.2	4.83 ± 0.09	20.8 ± 0.6	99.2 ± 0.3	49.0 ± 1.0
3ox1 3ox4-1	23.6 ± 0.2	4.96 ± 0.12	23.3 ± 0.4	95.8 ± 0.4	47.0 ± 0.6
3ox1 3ox4-3	20.8 ± 0.2	4.92 ± 0.09	23.0 ± 0.3	97.0 ± 0.4	49.3 ± 0.6
3ox1 3ox3	23.6 ± 0.2	4.88 ± 0.13	23.9 ± 0.6	59.9 ± 1.9	33.4 ± 1.0
3ox1 3ox3 3ox4-1	24.0 ± 0.2	5.13 ± 0.09	23.9 ± 0.3	54.7 ± 1.2	30.6 ± 0.6
3ox1 3ox3 3ox4-3	23.1 ± 0.2	5.07 ± 0.18	25.8 ± 0.3	51.2 ± 1.5	29.2 ± 0.7

Supplemental Table 2. Phenotype comparison of *ga3ox4-1* and *ga3ox4-3* in *ga3ox1* and *ga3ox1 ga3ox3* backgrounds.

Rosette diameter was measured 24 days after sowing. Silique data were collected from primary inflorescence stem only. Fertile siliques contain at least one seed. All measurements are the means ± S.E. from 15-20 plants per genotype.

	Flowering time (days)	Flowering time (rosette leaf #)	Rosette diameter (cm)	Final height (cm)	% of fertile siliques	Seed per fertile silique
WT	20.1 ± 1.2	9.1 ± 0.7	6.6 ± 0.8	40.9 ± 1.9	97.9 ± 2.2	57.2 ± 2.9
ga3ox2	18.8 ± 1.5	8.7 ± 0.5	7.1 ± 0.7	42.8 ± 4.5	98.5 ± 2.0	55.4 ± 3.8
ga3ox3	21.3 ± 1.1	9.4 ± 0.6	6.3 ± 0.7	41.4 ± 1.9	96.5 ± 2.4	54.3 ± 2.2
ga3ox4	22.4 ± 2.2	9.3 ± 0.5	6.7 ± 0.8	42.2 ± 2.3	97.2 ± 2.9	54.4 ± 4.1
ga3ox2 ga3ox3	22.4 ± 2.1	8.9 ± 0.5	6.1 ± 0.8	45.3 ± 4.1	98.3 ± 1.8	54.4 ± 5.9
ga3ox3 ga3ox4	20.6 ± 1.5	9.0 ± 0.8	7.3 ± 1.0	41.2 ± 4.0	97.9 ± 2.1	53.2 ± 5.4

Supplemental Table 3. Phenotypic characterization of category I *ga3ox* mutants in comparison to WT.

All measurements are the means ± S.E. from 15-20 plants per genotype. Rosette diameter was measured 24 days after sowing. Measurements of seeds in siliques were taken on primary inflorescence stems only. Fertile siliques contain at least one seed.

		ga3ox1 ga3ox3	*ga3ox1 ga3ox3 ga3ox4*	*ga3ox1 ga3ox2 ga3ox3*
% of fertile siliques	**#1-5 siliques**	2.7 ± 1.8	1.5 ± 1.4	0.0 ± 0.0
	#6-10 siliques	28.0 ± 5.8	18.5 ± 4.9	0.0 ± 0.0
	#11-15 siliques	14.7 ± 5.0	1.5 ± 1.4	0.0 ± 0.0
	#16-20 siliques	25.3 ± 8.4	12.3 ± 5.0	5.3 ± 3.1
	#21-25 siliques	72.0 ± 6.7	33.8 ± 6.1	41.3 ± 7.7
	#26-30 siliques	90.7 ± 2.7	81.5 ± 4.5	88.0 ± 4.3
	all siliques	59.9 ± 1.9	51.2 ± 1.5	45.8 ± 1.5
Seeds per fertile silique	**#1-5 siliques**	21.0 ± 20.0	1.0 ± 0.0	no fertile siliques
	#6-10 siliques	15.5 ± 3.3	11.9 ± 2.1	no fertile siliques
	#11-15 siliques	15.2 ± 4.0	13.0 ± 0.0	no fertile siliques
	#16-20 siliques	19.8 ± 5.7	10.0 ± 2.4	11.3 ± 6.3
	#21-25 siliques	25.1 ± 2.2	16.9 ± 2.5	20.7 ± 4.5
	#26-30 siliques	35.3 ± 2.2	19.5 ± 1.7	35.1 ± 2.6
	all siliques	33.4 ± 1.0	29.2 ± 0.7	32.8 ± 1.8

Supplemental Table 4. Comparison of fertility in *ga3ox1 ga3ox3*, *ga3ox1 ga3ox3 ga3ox4* and *ga3ox1 ga3ox2 ga3ox3* mutants.

Silique data were collected from primary inflorescence stem only. Fertile siliques contain at least one seed. All measurements are means ± S.E. from 13-15 plants per line.

Supplemental Table 5. List of Primers and Their Uses

Cloning

Primer	Sequence	Used for	Notes
5'3ox32kbprSalI	5'-ACGCGTCGACCATATCGAGTATGTCTTGGTGAACC	Cloning of the whole 1.8 kb *GA3ox3* promoter	For construction of *GA3ox3-TC-GUS*
3'3ox35'UTRBamHI	5'-CGCGGATCCCTGTGTGACAGAGCTCA**G**GATT		
5'3ox32kbprHindIII	5'-CCCAAGCTTCATATCGAGTATGTCTTGGTGAACC	Cloning of partial 1.2 kb *GA3ox3* promoter	For construction of *GA3ox3-TL-GUS*
3'3ox3pr630up	5'-GATATGCTTTGATAGTCAAATCAAG		
5'3ox3pr750up	5'-GGAAGCCTACTAACACGAGG	Cloning of 1.55 kb *GA3ox3* fragment including 0.6 kb promoter, first exon, intron and part of second exon,	For construction of *GA3ox3-TL1-GUS*
3'3ox3ex2BamHI	5'-CGCGGATCCCCCTAACGAGCCCATCAACAT		
3'3ox3ex3BamHI	5'-CGCGGATCCTCTCGACCCTTCTTCTTGTGG	Cloning of 2.3 kb *GA3ox3* fragment including 0.6 kb promoter, first two exons, first two introns and part of the third exon	For construction of *GA3ox3-TL2-GUS*
3ox3-Ex5-Xmn	5'-GGAAGGATTTCGATGAGCTCTGTCACACAG	Cloning of *GA3ox3* coding sequence	For construction of pMALc2-*GA3ox3*
3ox3-Ex3'	5'-GGTAAGCTTTCAGCAACGGAACAAAGT		
5'3ox41kbprSalI	5'-ACGCGTCGACCTGTCTTCCGACTGAATTTCTTG	Cloning of the whole 1.2 kb *GA3ox4* promoter	For construction of *GA3ox4-TC-GUS*
3'3ox45'UTRSmaI	3'-TCCCCCGGGGACTTGTGAACTAATGAGAGAATGGG		
3'3ox4ex2SmaI	5'-TCCCCCGGGAGACCCATGGCTCGTTCTGGTTCAGGACAAAC	Cloning of *GA3ox3* promoter, first exon, intron and part of second exon	For construction of *GA3ox4-TL-GUS*, with 5'3ox41kbprSalI
5'3ox4BamHIpMAL	5'-CGCGGATCCATGCCTTCACTAGCAGAAGAGATATG	Cloning of *GA3ox4* coding sequence	For construction of pMALc2-*GA3ox4*
3'3ox4SalIpMAL	3'-ACGCGTCGACTTAATTGGTGGGATTAACGACCC		

T-DNA, TILLING Analysis			
Primer	Sequence	Used for	Notes
3ox3F	5’-TGAGCTCTGTCACACAGCTATTCAAGAACAAC	Genotyping *GA3ox3*	WT allele 2 kb PCR product
3ox3R	5’-CGGAACAAAGTCAAGGCTTTGTTAAAGTGTG		
JMLB1	5’-GGCAATCAGCTGTTGCCCGTCTCACTGGTG	Genotyping *ga3ox3*	Mutant allele (with 3ox3F) 800 bp PCR product
3ox3Fint	5'-GCCGAGTTCTGCAATGTGATGGAAG-3'	RT-PCR of GA3ox3	cDNA product is 600 bp (with 3ox3R). DNA contamination has a 1.4 kb product
3ox4left	5’-CCGATGACTCCTACCGCAACCATT	*GA3ox4* TILLING analysis	
3ox4right	5’-CCTAATTGCGTCCATGGCCTTGTC		
CS94121F	5’-AGGCGCCATACGACTAAACCA**G**TAC	*ga3ox4-1* and *ga3ox4-4* genotyping	C to G mutation to generate dCAPs primer. PCR product is 305 bp. For *ga3ox4-1*, HinFI cuts WT allele only. For *ga3ox4-4*. ScaI cuts mutant allele only.
CS94121R	3’-CAGCTGGACCACCCCATAAGTACGC		
3ox45'UTR-2	5’-CTACAACCCATCATGACAAGTCTCATC	*G*enotyping *GA3ox4-2, ga3ox4-2*	WT allele 2.1 kb PCR product. Mutant allele (JMLB1+ 3ox4R) 1.8 kb PCR product
3ox4R	5’-TGGTGGGATTAACGACCCTAATTGCGTCC		
3ox4F	5’-ATGCCTTCACTAGCAGAAGAGATATGTATTGG	*G*enotyping *GA3ox4-3, ga3ox4-3*	WT allele (with 3ox4R) 1.5 kb PCR product; mutant allele (with JMLB1) 0.6 kb PCR product
Quantitative Applications			
Primer	Sequence	Used for	Notes
Roc1Frt	5’-ATCTACGGGAGCAAGT	*ROC1* qPCR	225 bp PCR product
Roc1Rrt	5’-GTCGGCTTTCCAGATG		

SUPPLEMENTAL METHODS

Absolute Expression Levels of *GA3ox* Genes

The standard curve of *ROC1* cDNA copy vs. cycle number is generated using linearized plasmid containing *ROC1* cDNA (pCG22; Lippuner et al., 1994), according to the same procedure as described in Tyler et al. (2004). The standard curve of cDNA copy vs. cycle number for each *GA3ox* gene was determined previously (Mitchum et al., 2006). Based on these standard curves, the cDNA copy numbers of *GA3ox* genes and *ROC1* can be determined according to their respective cycle numbers.

RT-PCR

Total RNAs were extracted from *ga3ox3* or WT siliques as described in Methods. RT-PCR was performed using rTth DNA polymerase (Roche Diagnostics, Mannheim, Germany), according to product manual. GA3ox3 intron-spanning primers (3ox3Fint + 3ox3R; Supplemental Table 5 online) were used in the reaction. PCR products were separated and visualized on 1.2% agarose gel.

Construction of *GA3ox-GUS* and MBP-GA3ox Plasmids

All plasmid constructs containing PCR-amplified DNA inserts were sequenced to confirm that no mutations were introduced by PCR. Primer sequences are listed in Supplemental Table 5 online.

For making the GA3ox3-GUS plasmids, genomic DNA fragments for *GA3ox3* were amplified by PCR from *Arabidopsis* BAC Clone F17L22. Primers containing *Sal*I and *Bam*HI restriction sites (5'3ox32kbpr*Sal*I and 3'3ox35'UTR*Bam*HI) were used to amplify 1789 nucleotides of the *GA3ox3* promoter region for directional cloning into binary vector pBI101.1 upstream of the *GUS* reporter gene to generate p3ox3-TC-GUS. A three-step cloning process was used to construct p3ox3-TL1-GUS and p3ox3-TL2-GUS taking advantage of a native *Hind*III restriction site at position -721 in the *GA3ox3* promoter region. First, primer 5'3ox3750up and either 3'3ox3ex2*Bam*HI or 3'3ox3ex3*Bam*HI were used to generate 1548 and 2292 bp fragments, respectively. The fragments were digested with *Hind*III and *Bam*HI and directionally cloned into the binary vector pBI101.1 to generate plasmid constructs 3ox3pr2kb-

Ex2 and 3ox3pr2kb-Ex3, respectively. In step two, primers 5'3ox32kbpr*Hind*III and 3'3ox3pr630up were used to amplify a 1191 bp fragment. The fragment was digested with *Hind*III and cloned into pBluescript SK+ to generate plasmid construct 3ox3prHindIII. The 1191bp *Hind*III fragment from 3ox3prHindIII was subcloned into 3ox3pr2Kb-Ex2 to generate p3ox3-TL1-GUS and 3ox3pr2kb-Ex3 to generate p3ox3-TL2-GUS.

For making the GA3ox4-GUS plasmids, genomic DNA fragments for *GA3ox4* were amplified by PCR from *Arabidopsis* BAC Clone F5I6. Primers containing *Sal*I and *Sma*I restriction sites (5'3ox41kbpr*Sal*I and 3'3ox45'UTR*Sma*I) were used to amplify 1157 nucleotides of the *GA3ox4* promoter region for directional cloning into binary vector pBI101.1 upstream of the *GUS* reporter gene to generate p3ox4-TC-GUS. Primers containing *Sal*I and *Sma*I restriction sites (5'3ox41kbpr*Sal*I and 3'3ox4ex2*Sma*I) were used to amplify 1157 nucleotides of the *GA3ox4* promoter region, exon 1, intron 1, and 201 nucleotides of exon 2 for directional cloning into binary vector pBI101.1 to generate p3ox4-TL-GUS.

For making the MBP-GA3ox expression constructs for in vitro enzyme assays, total RNA was isolated from siliques of 35-day-old plants of WT, *ga3ox4-1*, and *ga3ox4-4* using the RNeasy Kit and subjected to on-column DNase treatment (Qiagen, Valencia, CA). The First Strand cDNA Synthesis Kit for RT-PCR (Roche Diagnostics, Mannheim, Germany) was used to make cDNA from 1 µg of RNA. Primers containing *Bam*HI and *Sal*I restriction sites (5'3ox4*Bam*HIpMAL and 3'3ox4*Sal*IpMAL) were used to amplify and directionally clone the WT and mutant forms of GA3ox4 into the *E.coli* expression vector pMALc2 (New England Biolabs, Beverly, MA). Primers containing *Xmn*I and *Hind*III restriction sites (3ox3-Ex5-Xmn and 3ox3 Ex3') were used to amplify and directionally clone *GA3ox3* into pMALc2.

Identification of *ga3ox4* Alleles by TILLING

The *ga3ox4-1* and *ga3ox4-4* mutants were identified via the *Arabidopsis* TILLING (Targeting Induced Local Lesions in Genomes) Project (Henikoff et al., 2004). Cy-3 and Cy-5 labeled oligonucleotide primers (3ox4left and 3ox4right, Supplemental Table 5 online) spanning 1047 nucleotides (410-1456) of the *GA3ox4* gene were used to screen for *ga3ox4* mutations at the *Arabidopsis* TILLING Center (University of Washington). M3 seeds corresponding to two mutants with predicted deleterious mutations were requested from the TILLING center and characterized in more detail. CS91343 (*ga3ox4-1*) had a G-to-A change at nucleotide 1094.

CS94121 (*ga3ox4-4*) had a C-to-T substitution at nucleotide 1046. Homozygous mutant plants were identified by PCR using allele-specific dCAPs primers (CS94121F+CS94121R; Supplemental Table 5 online) designed using the dCAPs Finder 2.0 Website (http://helix.wustl.edu/dcaps/dcaps.html; Neff et al., 2002). The *ga3ox4-1* allele was backcrossed three times to remove the *erecta* allele that was present in the parental background, and additional mutations induced by EMS.

Scanning Electron Microscopy

Col-0 and *ga3ox4* mutant seeds were first coated with gold/platinum (60%/40%) in a sputter coater (Hummer 6.2, Anatech, Ltd., Springfield, VA) and then visualized using an ESEM (Philips XL30 ESEM TMP, FEI Company, Hillsboro, OR) at 25 kV. Images were captured by the SEM integrated camera.

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