

Supplemental Figure 1. Phenotypic Analysis of Reproductive Organs in WT and GA-Related Mutants.

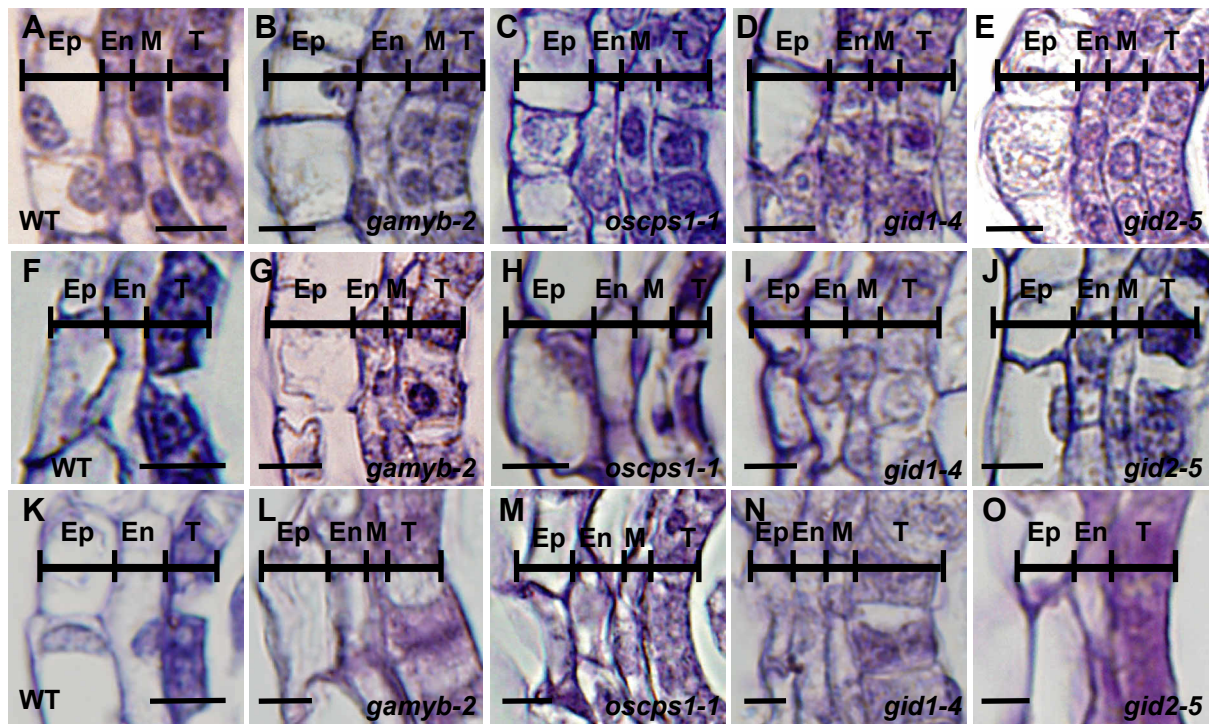
(A) Gross morphology of WT, *gamyb-2*, and GA-related mutants (*oscps1-1*, *gid1-4*, *gid1-7*, and *gid2-5*) at 2 months after sowing. Bar = 20 cm.

(B) Flowers of the WT, *gamyb-2*, and GA-related mutants.

Cp, carpel; Le, lemma; Lo, lodicule; PI, palea; St, stamen. Bar = 2 mm.

(C) Stamens of the WT, *gamyb-2*, and GA-related mutants. An, anther; FI, filament. Bar = 1 mm.

(D) Pistils of the WT, *gamyb-2*, and GA-related mutants. Bar = 1 mm.



Supplemental Figure 2. Higher Magnification of Transverse Section from WT and GA-Related Mutants.

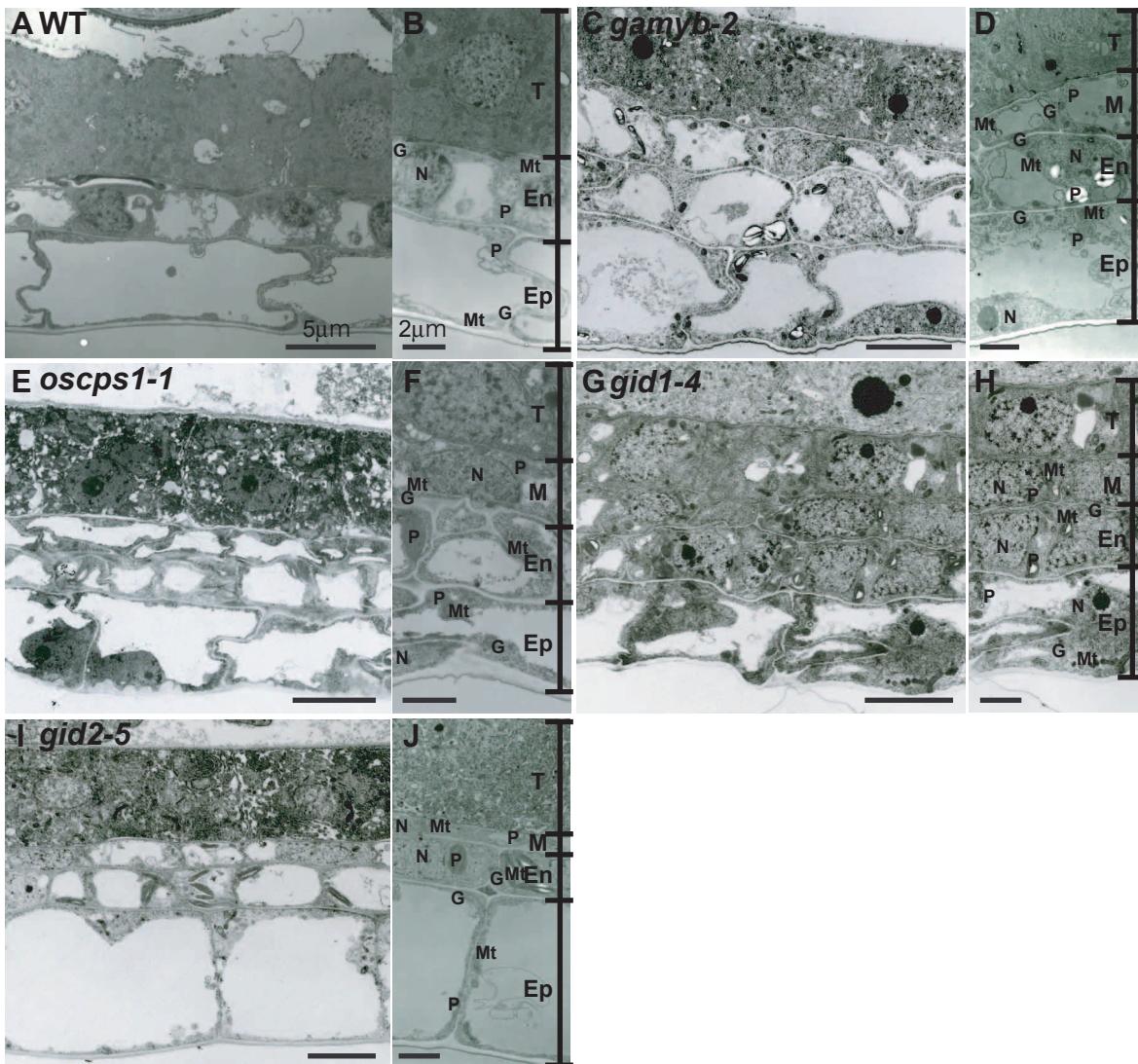
Anther development in WT and GA-related mutants was compared at the three different stages: pollen mother cell (PMC), meiosis (MEI), and tetrad (TD).

(A) to (E) Transverse section of anthers at the PMC stage in WT **(A)**, *gamyb-2* **(B)**, *oscps1-1* **(C)**, *gid1-4* **(D)**, and *gid2-5* **(E)**.

(F) to (J) Transverse section of anthers at the MEI stage in WT **(F)**, *gamyb-2* **(G)**, *oscps1-1* **(H)**, *gid1-4* **(I)**, and *gid2-5* **(J)**.

(K) to (O) Transverse section of anthers at the TD stage in WT **(K)**, *gamyb-2* **(L)**, *oscps1-1* **(M)**, *gid1-4* **(N)** and *gid2-5* **(O)**.

Ep, epidermal cell layer; En, endothelial cell layer; M, middle layer; T, tapetal layer. Bars = 5 μ m.

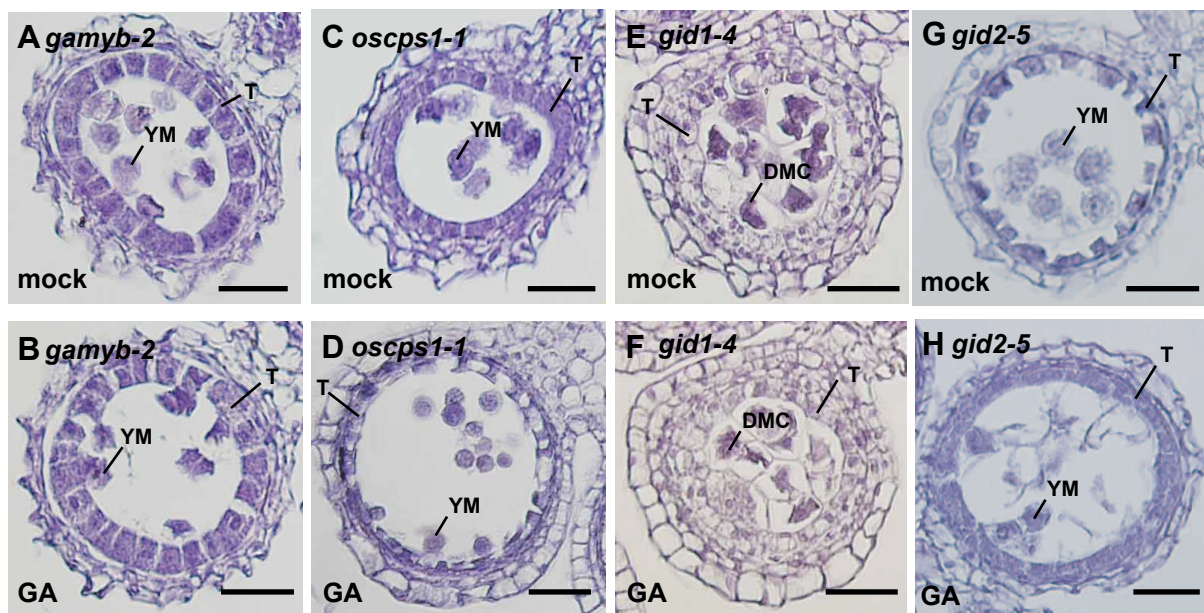


Supplemental Figure 3. Ultrastructural Analysis of the Anther Wall Layers in WT and GA-Related Mutants by TEM.

(A) to (F), (I) and (J) The anther wall layers at the YM stage in WT ([A] and [B]), *gamyb-2* ([C] and [D]), *oscps1-1* ([E] and [F]), and *gid2-5* ([I] and [J]).

(G) and (H) The anther wall layers at the MEI stage in *gid1-4*.

(B), (D), (F), (H), and (J) Higher Magnification for each anther layers with indications of cellular organs. N, nucleus; P, plastid; Mt, mitochondria; G, Golgi body; Ep, epidermal cell layer; En, endothelial cell layer; M, middle layer; T, tapetal layer.



Supplemental Figure 4. Histological Analyses of GA₃-Treated Anthers in GA-Related Mutants.

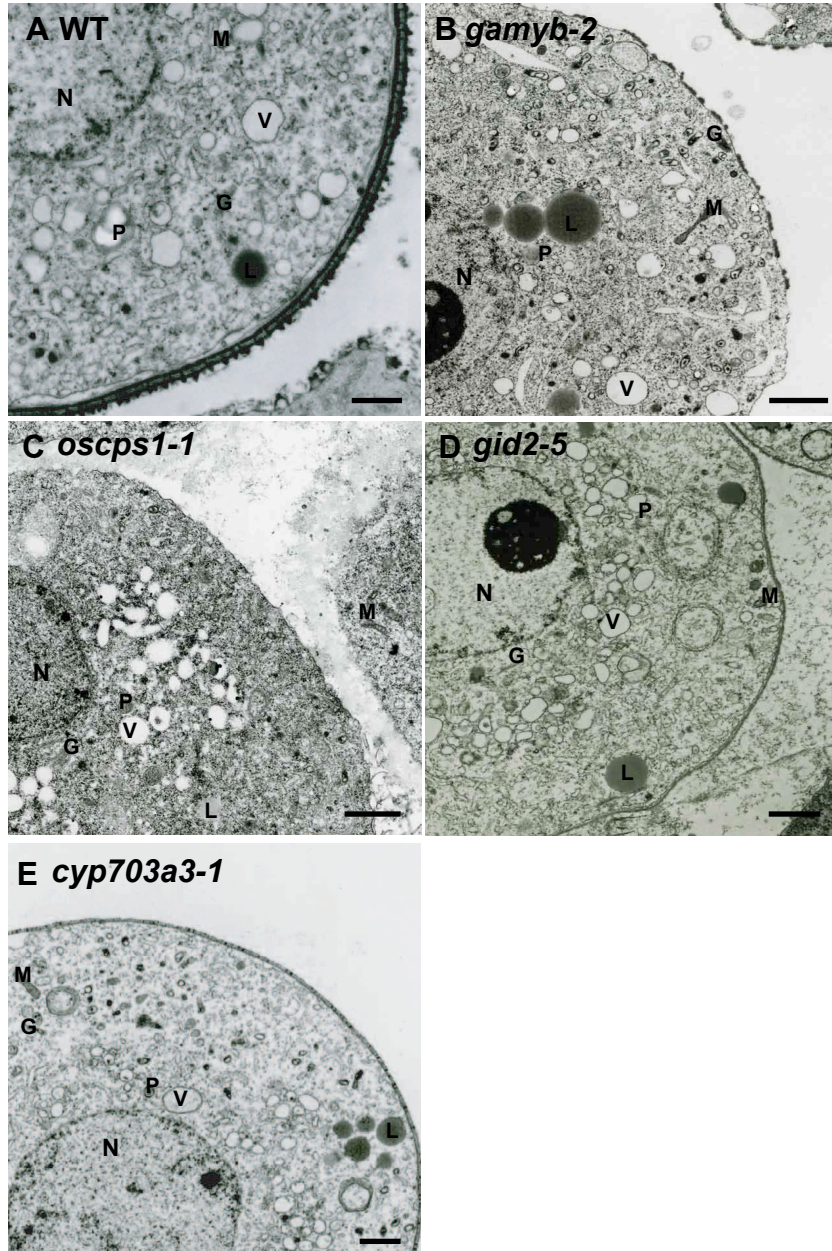
(A) and **(B)** Transverse section of anther at the YM stage in *gamyb-2*, treated by mock (0.1 % ethanol) **(A)** or 10⁻⁵ M GA₃ **(B)**.

(C) and **(D)** Transverse section of anther at the YM stage in *oscps1-1*, treated by mock (0.1 % ethanol) **(C)** or 10⁻⁵ M GA₃ **(D)**.

(E) and **(F)** Transverse section of anther at the TD stage in *gid1-4*, treated by mock (0.1 % ethanol) **(E)** or 10⁻⁵ M GA₃ **(F)**.

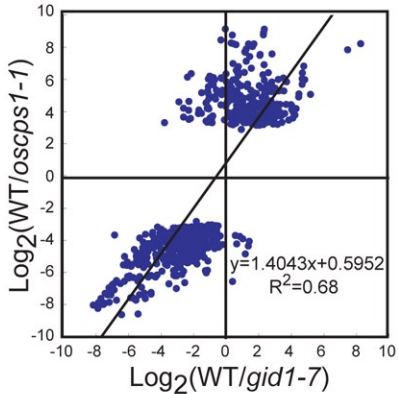
(G) and **(H)** Transverse section of anther at the YM stage in *gid2-5*, treated by mock (0.1 % ethanol) **(G)** or 10⁻⁵ M GA₃ **(H)**.

YM, young microspore; T, tapetal layer; DMC, degraded meiocyte. Bars = 25 μm.



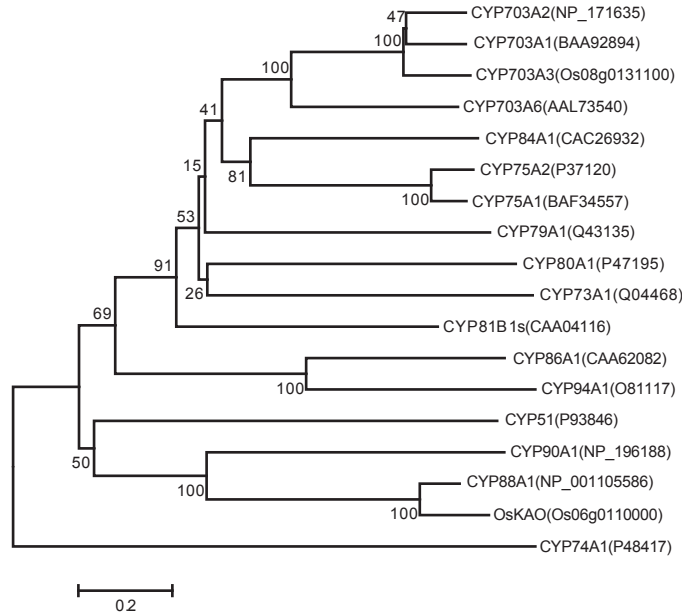
Supplemental Figure 5. The cytoplasm of Microspores in WT, GA-Related Mutants, and *cyp703a3-1* by TEM.

(A) to (E) Ultrastructure of the cytoplasm in WT (A), *gamyb-2* (B), *oscps1-1* (C), *gid2-5* (D), and *cyp703a3-1* (E). N, nucleus; P, plastid; M, mitochondria; G, Golgi body; V, vacuole; L, lipid body. Bars = 1 μ m.



Supplemental Figure 6. Scatter Plot Analysis to Compare Genes Regulated by GA (*oscps1-1* background) and GID1 in Anther.

Axes show the log_2 value of the ratios of signal intensities observed in the indicated plants.

A**B**

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CYP703A1 1 MIDFTNYLLVLFPRVYIFYLVDYALIFVVCVYLVSGLVHFSFIEISKQKINRLPFGKQWPI
CYP703A2 1 -----MILVLSLPAVLILNVLWRRLASAKAQRLLPFGPFRWPI
CYP703A3 1 -----MDFPFLSLILCSWIFVWVSWKLNQMRRLPFGPFRWPI
CYP703A6 1 -----MDFPFLSLILCSWIFVWVWRRLNMRRLPFGPFRWPI

CYP703A1 61 VGNLLQLGQLPFRHDMASFCDKYGFLVYLRGNVDAITTDPEHIREILVQDDIFASRFR
CYP703A2 42 LGNLLQLGFLPFRDLASLCDKYGFLVYLRGNVDAITTDPEHIREILVQDDIFASRFR
CYP703A3 40 FGNLLQLGFLPFRHDFARFCIKYGFLVYLRGLTDAITTDPEVIREILVQDDIFASRFR
CYP703A6 40 FGNLLQLGFLPFRHDFARFCIKYGFLVYLRGLTDAITTDPEVIREILVQDDIFASRFR

CYP703A1 121 FLAAVHLLAYGCGDVALAPLGPWKRMRRCMEHLLTKRRLSEFGMHRADAEQSLVEDVWA
CYP703A2 102 FLAAVHLLAYGCGDVALAPLGPWKRMRRCMEHLLTKRRLSEFTTQRAEARYLIRDVFK
CYP703A3 100 FLAAVHLLAYGCGDVALAPLGPWKRMRRCMEHLLTKRRLSEFAAHRADAEHLLCQVWA
CYP703A6 100 FLAAVHLLAYGCGDVALAPLGPWKRMRRCMEHLLTKRRLSEFAAHRADAEHLLCQVWA

CYP703A1 181 KTSKSEIVNLPDILGAFSMNNVTRMLLGGKQVFC-ALSGAGGEAMEFMHITHELFWLLGVI
CYP703A2 162 RSEKSEIVNLPDILGAFSMNNVTRMLLGGKQVFC-PGSLVSEKAGSEFMHITHELFWLLGVI
CYP703A3 160 KTSKSEIVNLPDILGAFSMNNVTRMLLGGKQVFC-LQSGAGGEAMEFMHITHELFWLLGVI
CYP703A6 160 KTSKSEIVNLPDILGAFSMNNVTRMLLGGKQVFC-IQSGAGGEAMEFMHITHELFWLLGVI

CYP703A1 240 YLGDYLPFRWRHDPHGCEKKMRVEKRVDDFHQKIIDEHRRAREAKKS-----GKNVDE--G
CYP703A2 222 YLGDYLPFRWRHDPHGCEKKMRVEKRVDDFHQKIIDEHRRAREAKKS-----KLEDEKNG
CYP703A3 219 YLGDYLPFRWRHDPHGCEKKMRVEKRVDDFHQKIIDEHRRAREAKKS---ASLDDDNKE
CYP703A6 219 YLGDYLPFRWRHDPHGCEKKMRVEKRVDDFHQKIIDEHRRAREAKKTRRSLEDDDDGKE

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CYP703A2 273 DMDFVDVLLSLPGE----NGKQHMDDVEIKALIQDMIAAATDTSVNTNEWMAEVIKRF
CYP703A3 276 DMDFVDVLLSLPGE----NGKEHMDDVEIKALIQDMIAAATDTSVNTNEWMAEVIKRF
CYP703A6 279 DMDFVDVLLSLPGE----NGKEHMDDVEIKALIQDMIAAATDTSVNTNEWMAEVIKRF

CYP703A1 349 NVLNRKIQBEELDIVVGSDRMVESDLVHLKYLRCVVRSEFRMHFAGFPFLIPHESIRDTKIN
CYP703A2 328 RVNRKIQBEELDIVVGSNRMVESDLVHLKYLRCVVRSEFRMHFAGFPFLIPHESVRAATTIN
CYP703A3 331 NVLNRKIQBEELDIVVGRMRVAESDLGQLTYLRCVVRSEFRMHFAGFPFLIPHESLRPTTIM
CYP703A6 334 NVLNRKIQBEELDIVVGRMRVAESDLGQLTYLRCVVRSEFRMHFAGFPFLIPHESLRPTTIM

CYP703A1 409 GYIFPARTRVFINTHGLGRNTKIWDNIDFRPERHLPAGEE-SRVEISHGADFKILPFGSA
CYP703A2 388 GYIFPARTRVFINTHGLGRNTKIWDNIDFRPERHLPAGEE-SRVEISHGADFKILPFGSA
CYP703A3 391 GHVIFPARTRVFINTHGLGRNTKIWDNIDFRPERHLPAGEE-SRVEISHGADFKILPFGSA
CYP703A6 394 GHVIFPARTRVFINTHGLGRNTKIWDNIDFRPERHLPAGEE-SRVEISHGADFKILPFGSA

CYP703A1 469 GRRKCPGAPLGVILVLMALARLPHCFDWSPPDGLRPEDIDTIEVYGMTMPKAKPLVAVAT
CYP703A2 447 GRRKCPGAPLGVILVLMALARLPHCFDWSPPDGLRPEDIDTIEVYGMTMPKAKPLVAVAT
CYP703A3 451 GRRKCPGAPLGVILVLMALARLPHCFDWSPPDGLRPEDIDTIEVYGMTMPKAKPLVAVAT
CYP703A6 453 GRRKCPGAPLGVILVLMALARLPHCFDWSPPDGLRPEDIDTIEVYGMTMPKAKPLVAVAT

CYP703A1 528 PRLFDHLVHSIK---
CYP703A2 502 PRLAAHLVY-----
CYP703A3 511 PRLPFDHLYGRHGKQV
CYP703A6 513 PRLPFDHLYGGGSAS-

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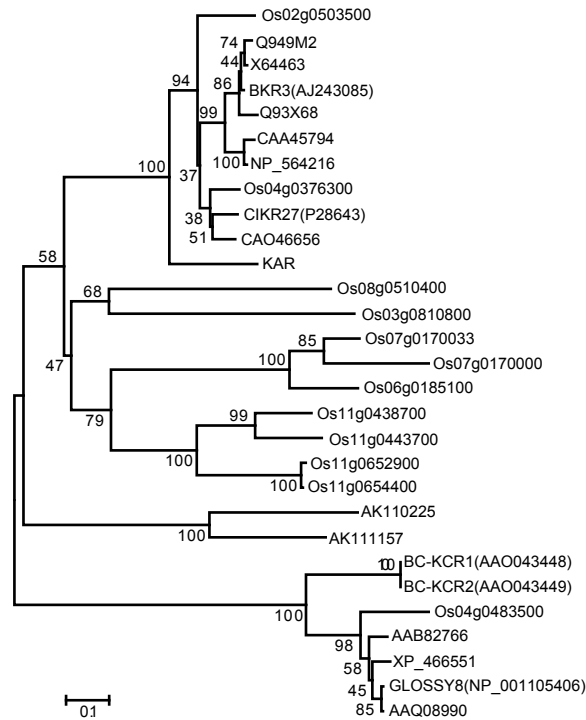
Supplemental Figure 7. Structure of CYP703A3.

(A) Phylogenetic tree of the cytochrome P450 proteins.

CYP703A3 belongs to the same group as CYP703A1, CYP703A2, and CYP703A6, which are involved in lipid metabolism as a lauric acid in-chain hydroxylase. The phylogenetic tree was generated using MEGA 4.0 with the neighbor joining method. Bootstrap values were calculated from 1,000 trials and are shown at each node. The extent of divergence according to the scale (relative units) is shown at bottom.

(B) Alignment of the amino acid sequences of CYP703A-family proteins.

The alignment was generated using CLUSTALX 2.0 with default parameters and BoxShade 3.21. Positions of identical and similar sequences are boxed in black and grey, respectively.

A**B**

CIKR27	1	M A T A T A A G - C S G A V A L K S L G G R R L C I P O Q L S P V L A G F G S H A A K S F P I L S T R - S I A T S G - I
BKR3	1	M A T T V A A T K L T S L K A V K K L G F R E I R Q V R Q W T P L Q S ----- S M P H F G S R Q S F A T S T V V
KAR	1	--- M A S A A Y Y A G L V A G T P P P P C R P G R L R R C Q P P A N----- N L V S S G R R
CIKR27	58	R A Q V A T A E K - V S A G A G O S V E S P V V I V T G A S R G I G K A I A L S L G K A G C K V L V N Y A R S S K E A E
BKR3	53	K A Q A T A V E Q - S T G E A V P K V E S P V V V V T G A S R G I G K A I A L S L G K A G C K V L V N Y A R S A K E A E
KAR	41	S H Q A I R V T N G V N M D G R A K L A A P V A V V T G A S R G I G R A I A V A L G K A G C K V I V N Y A K S G M E A E
CIKR27	117	E V S K E I E I A F G G Q A L T F G G D V S K E E D V E A M I K T A V D A W G T V D I L V N N A G I T R D G L L M R M K K
BKR3	112	E V S K Q I E I A Y G G Q A I T F G G D V S K E A D V E A M M K T A I D A W G T I D V V V N N A G I T R D T L L I R M K K
KAR	101	E V C R E I E I E S G G T A I T F S A D V S I E A E V E S M M R A A I D T W G T L D V L V N N A G I T R D A L L M R M K R
CIKR27	177	S Q W Q E V I D L N L T G V F L C T Q A A A K I M M K K K G R I I N I A S V V G L V G N A G Q A N Y S A A K A G V I G
BKR3	172	S Q W D E V I D L N L T G V F L C T Q A A T K I M M K K R K G R I I N I A S V V G L I G N I G Q A N Y A A A K A G V I G
KAR	161	T Q W Q E V V D V N L T G V V L C A Q A A A G V M M M K K K G R I I N I T S V S G I I G N I G Q A N Y C A A K A G V I G
CIKR27	237	F T K T V A R E Y A S R N I N V N A V A P G F I S S D M T S K L G D D I N K K I L E T I P L G R Y G Q P E E V A G L V E
BKR3	232	F S K T A A R E G A S R N I N V V C P G F I A S D M T A K L G E D M E K K I L G T I P L G R Y G Q P E D V A G L V E
KAR	221	L T K A M A R E Y G S R N I N V N A V A P G W V T S N M T A K L G D N V E Q K A L E T I P L G R F G K P E E I A G L V E
CIKR27	297	F L A I N P A S S Y V T G Q V F T I D G G M T M
BKR3	292	F L A L S P A A S Y I T G Q A F T I D G G I A I
KAR	281	F L A V H P A A S Y I T G Q V L P V D G G L S I

Supplemental Figure 8. Structure of KAR.**(A)** Phylogenetic tree of the KAR family proteins.

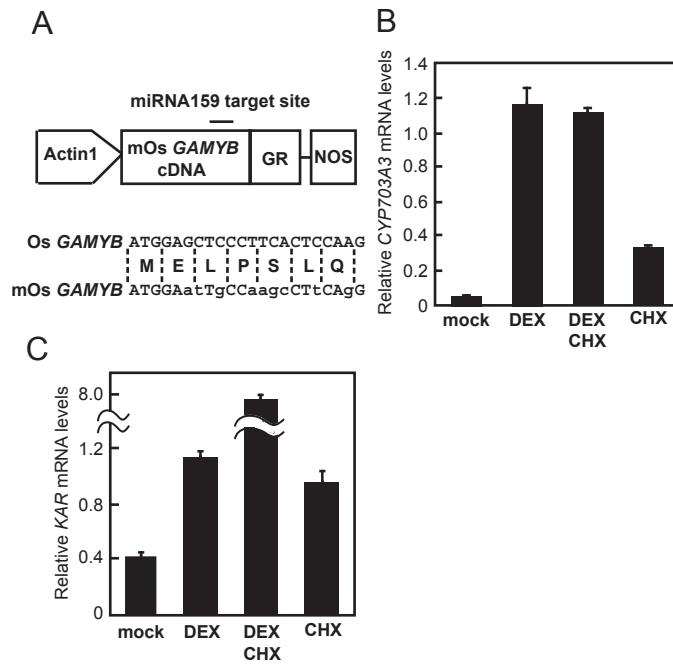
The phylogenetic tree was generated using MEGA 4.0 with the neighbor joining method.

Bootstrap values were calculated from 1,000 trials and are shown at each node. The extent of divergence according to the scale (relative units) is shown at bottom.

(B) The alignment of the amino acid sequences for CIKR27, BKR3, and KAR.

The alignment was generated using CLUSTALX 2.0 with default parameters and BoxShade 3.21. Positions of identical and similar sequences are boxed in black and grey, respectively.

Asterisks show the conserved catalytic triad for short-chain-alcohol reductase family.



Supplemental Figure 9. Expression Analysis for *CYP703A3* and *KAR* Genes Using the DEX-Inducible mOs *GAMYB-GR* Transgenic *gamyb-2* Plant.

(A) *ProAct1:mOs GAMYB-GR* construct. mOs *GAMYB-GR* fusion gene was driven by the *Actin1* promoter. mOs *GAMYB-GR* was consisted of the full-length Os *GAMYB* cDNA with nine synonymous nucleotide substitutions at the miRNA159 target site (lower letters in the bottom sequence), and the rat glucocorticoid receptor (GR).

(B) and **(C)** Real-time RT-PCR analysis of *CYP703A3* **(B)** and *KAR* **(C)** mRNAs. Total RNAs were isolated from the *gamyb-2* flowers carrying *ProAct1:mOs GAMYB-GR* treated with or without 10 μ M DEX and 10 μ M CHX for 4 h. The level of *CYP703A3* and *KAR* mRNA was normalized to *ACTIN1*. Data represent averages of three biological replicates, with error bars representing SD.



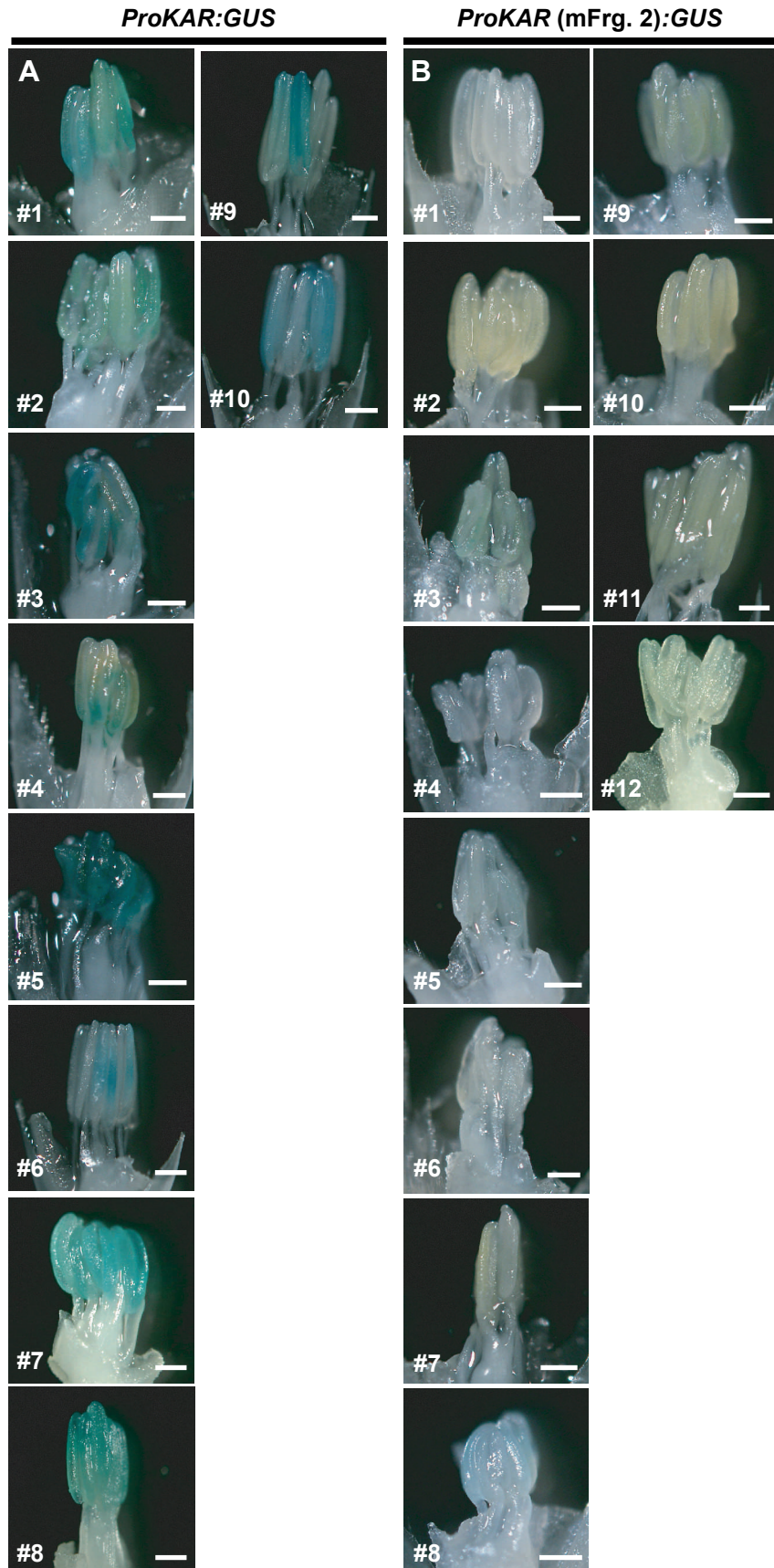
Supplemental Figure 10. GUS Activity of Stamens in All Transgenic Plants Carrying the Native or Mutagenized *ProCYP703A3*:GUS construct.

(A) Stamens from 10 T_0 transgenic lines with the *ProCYP703A3*:GUS construct.

(B) Stamens from 12 T_0 transgenic lines with the *ProCYP703A3* (mFrg. 2):GUS construct.

(C) Stamens from 18 T_0 transgenic lines with the *ProCYP703A3* (mFrg. 3):GUS construct.

Bars = 300 μ m.

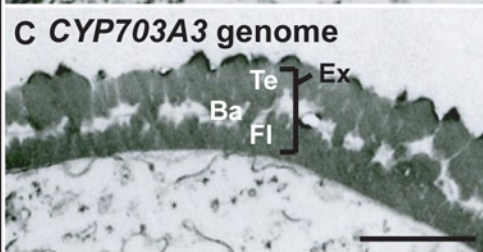
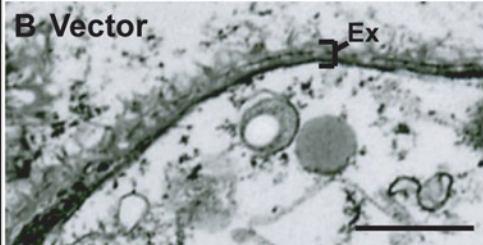


Supplemental Figure 11. GUS Activity of Stamens in All Transgenic Plants Carrying the Native or Mutagenized *ProKAR:GUS* Construct.

(A) Stamens from 10 T_0 transgenic lines with the *ProKAR:GUS* construct.

(B) Stamens from 12 T_0 transgenic lines with the *ProKAR (mFrg. 2):GUS* construct.

Bars = 300 μm .

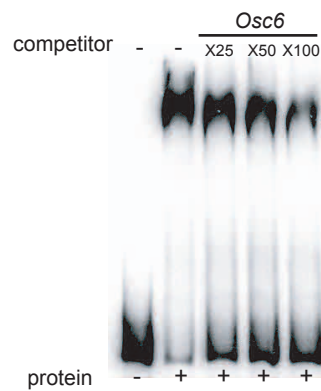


Supplemental Figure 12. Complementation Test of *cyp703a3-1*.

(A) Flowers of *cyp703a3-1* transgenic lines carrying empty vector or *CYP703A3* gene. Bar = 1 cm.

(B) and **(C)** Exine layers of *cyp703a3-1* transgenic lines carrying vector **(B)** or *CYP703A3* gene **(C)**. Ex, exine; Ba, bacula; Te, tectum; Fl, foot layer.

Bars = 1 μ m.



Supplemental Figure 13. Competitive Gel-Shift Assay with *Osc6* Promoter Region. Interaction between Os GAMYB and ^{32}P -labelled *RAMy1A* probe (-380 to -85) was efficiently competed by the fragment containing the GAMYB binding-like motif from *Osc6*. Competition experiments were performed using increasing molar amounts (X25, X50, X100) of the indicated unlabelled fragment; Lane 3 to 5, *Osc6* (-315 to -247).

Supplemental Table 1. PCR Primers Used in This Study

Gene name or vector name	Primer name	Sequence (5'-3')	Used for
TOS17	LTR4A	ACTGTATAGTTGGCCCATGTCCAG	genotyping for <i>cyp703a3-1</i> and <i>gamyb-2</i>
CYP703A3	AK106843-19U AK106843-19L	GTCATCCGTGAGATACTCATC ACCTCTCTGAGATTCACAGG	genotyping of <i>cyp703a3-1</i>
OsGAMYB	OsGAMYB-12U OsGAMYB-11L	TCAGCTCTCCAAAGTTTCCC CAGGTTCCATATTTAGGCCCC	genotyping of <i>gamyb-2</i>
CYP703A3	CYP703A3-SacIU CYP703A3-SmaIL	GAGCTCCTAAGTGCAGTGCGGTGGTT CCCGGGGACACCGATGATGTGTGCGGTGG	ProCYP703A3::GUS construct
KAR	KAR-SpeIU KAR-EcoRVL	ACTAGTGCGGAAGTGAATAACATGTAC GATATCGAATCCATGTTCACTCCATTGG	ProKAR::GUS construct
GUS	GUS-ClaIU GUS-ClaIL	ATCGATATGTTACGCTCTGTAGAAAC ATCGATTCAITGTTTGCCCTCCCTGCTG	OsGAMYB-GUS construct
CYP703A3	CYP703A3-mMYB1U CYP703A3-mMYB1L	TGTTACGAGAGATGTTGAACAA CATCTCTCGTAACATCATATAATA	ProCYP703A3 (mFrg. 2) ::GUS construct
CYP703A3	CYP703A3-mMYB2U CYP703A3-mMYB2L	TCTGTTCCGAATTCGCTTCAAC ATTGCGAACAGAAATTGACTGG	ProCYP703A3 (mFrg. 3) ::GUS construct
KAR	KAR-mMYBU KAR-mMYBL	TTCACGAACACACTCTTACTATC GTGTGTTTCGTAATGGTTAAACATG	ProKAR (mFrg. 2) ::GUS construct
Lipid transporter	AK119794-RTU AK119794-RTL	GCCATCAGCATCATGAACAG AACGAGACACGAAGACAACG	semiquantitative RT-PCR
OsMale Sterile 2	OsMS2-RTU OsMS2-RTL	GCAGCATCTACCAGCCCTAC TCCGCAATATCTTCTCGATG	semiquantitative RT-PCR
CYP703A3	AK106843-RTU AK106843-RTL	GCTAGGGAGGCCAAGAAGAG TTGGTCACCGATGATGTGTC	semiquantitative RT-PCR and real-time PCR
KAR	AK109188-RTU AK109188-RTL	ACATGACCGCAAAACTAGGC ATTGACAGGCCACCATCAAC	semiquantitative RT-PCR
Aspartic protease	AK105952-RTU AK105952-RTL	GGATGGTGTTCACACTTCCAG TTTTTCAGTTGACACGTGGTG	semiquantitative RT-PCR
Meiotic serine protease	AK106823-RTU AK106823-RTL	TCATCACTGACCGTCTCTCCG CCGAACTTGGTGACTCTCC	semiquantitative RT-PCR
OsActin1	Actin1-1 Actin1-2	CATCTTGGCATCTCTCAGCAC AACTTGTCCACGCTAATGAA	real-time PCR
OsActin1	Actin1-RTU Actin1-RTL	TCCATCTTGGCATCTCTCAG GTACCCTCATCAGGCATCTG	semiquantitative RT-PCR
Lipid transporter	AK119794-comU AK119794-comL	AATCATAACCGATTTCAATAG CGGGCCCGGTGTAACAAAC	DNA fragment of <i>lipid transporter</i> promoter region
OsMale Sterility 2	AK121254-comU AK121254-comL	TATACGTCATTTTTTTTACC GTTCCCTGGGGCCGCCAAAG	DNA fragment of <i>OsMale Sterility 2</i> promoter region
CYP703A3	AK106843-com1U AK106843-com3L	GAAGCCAGTTCATGAGTCATG CCATGAGCATAAGATGATGG	DNA fragment of CYP703A3 promoter region
KAR	AK109188-com1U AK109188-com2L	CGGTTGGTGTACCAACCCGG GTTTTCTTACCGTATGGGATC	DNA fragment of KAR promoter region
Aspartic protease	AK105952-comU AK105952-comL	TCCCCTTCGATTATTGTTAC GCGAAAGCTGTGCACGAAAG	DNA fragment of <i>Aspartic protease</i> promoter region
Meiotic serine protease	AK106823-comU AK106823-comL	GATTCTGTCCATGTAGATTCA CTATCTCCATGGGAGCTTCG	DNA fragment of <i>Meiotic serine protease</i> promoter region
RAmy1A	RAmy1A-comU RAmy1A-comL	AAATAGTTAATACTCAATTA TGGAGGCCTGGCTGGGCTTG	DNA fragment of <i>RAmy1A</i> promoter region
CYP703A3	AK106843-Frg1U AK106843-Frg1L	GAAGCCAGTTCATGAGTCATG CAATGTGCTCAGTCAATTC	DNA fragment (Frg. 1) of CYP703A3 promoter region
CYP703A3	AK106843-Frg2U AK106843-Frg2L	GAATTGCACTGAGCACATTG CATGATTCTTGTGCTGATC	DNA fragment (Frg. 2) of CYP703A3 promoter region
CYP703A3	AK106843-Frg3U AK106843-Frg3L	GATCAGCACAAGAATCATG CCATGAGCATAAGATGATGG	DNA fragment (Frg. 3) of CYP703A3 promoter region
KAR	AK109188-Frg.1U AK109188-Frg.1L	CGGTTGGTGTAAACACCCGG CTGAATGGTTAAACATGCAG	DNA fragment (Frg.1) of KAR promoter region
KAR	AK109188-Frg.2U AK109188-Frg.2L	CTGCATGTTTAAACCATTGAG GTTTTCTTACCGTATGGGATC	DNA fragment (Frg.2) of KAR promoter region
Osc6	AK064672-comU AK064672-comL	TAAACATGTGCTAAAAAGTC ATTTTCAACAACATACTCCCTC	DNA fragment of <i>Osc6</i> promoter region
GAMYB	GAMYB-XbaIU GAMYB-SmaIL	TCTAGAATGTATCGGGTGAAGAGCG CCCGGGTTTGAATCTGACATTTAC	ProAct1:mOs GAMYB-GR construct
GAMYB	GAMYB-mRNA159U GAMYB-mRNA159L	ATGGAATGCCAAGCCTTCAGGATACTGAAT CCTGAAGGCTTGGCAATTCATCTTCAAAG	ProAct1:mOs GAMYB-GR construct
pCR4 Blunt-TOPO vector	M13F M13R	GTAACACGACGGCCAG CAGGAAACAGCTATGAC	Sequencing and amplification