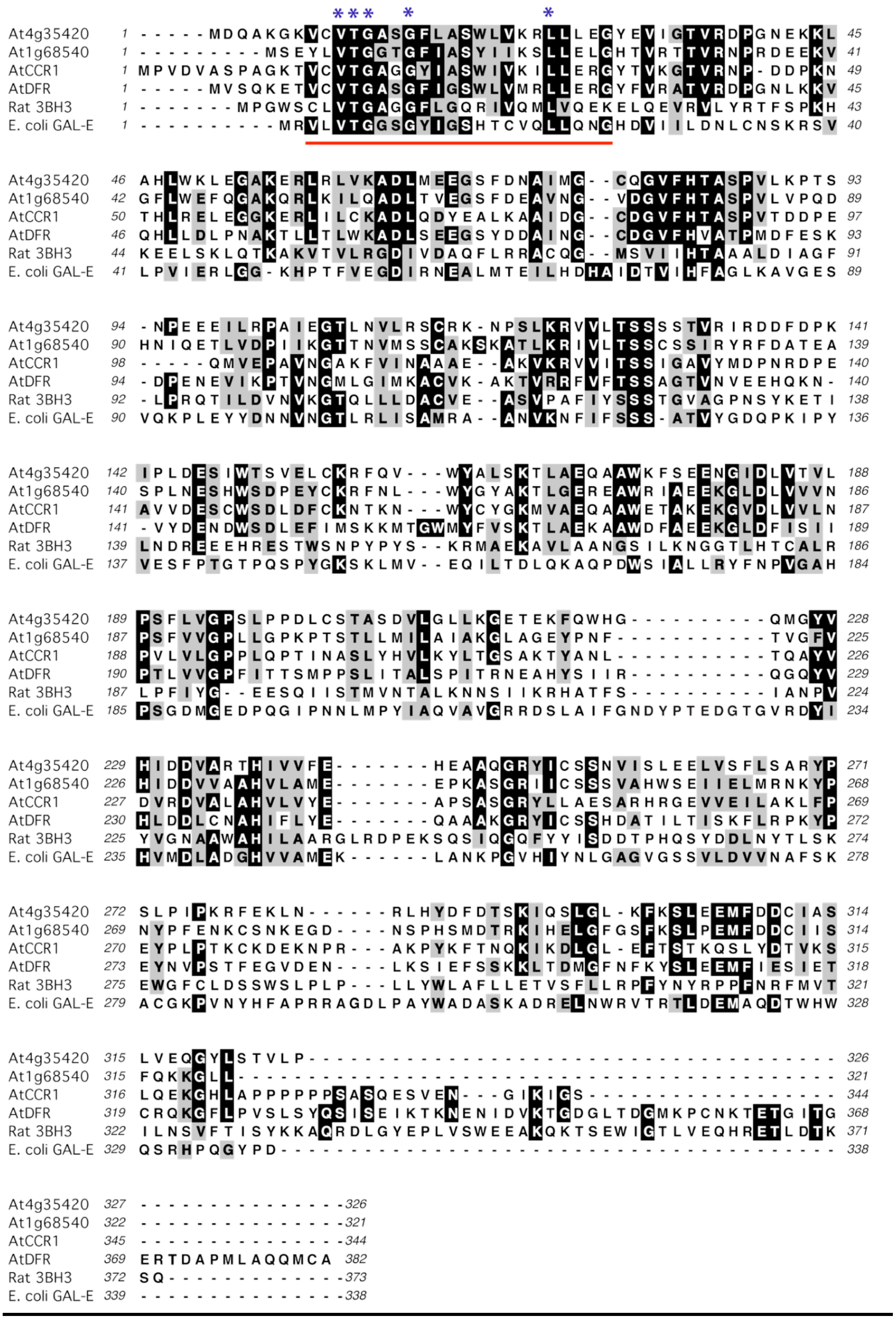
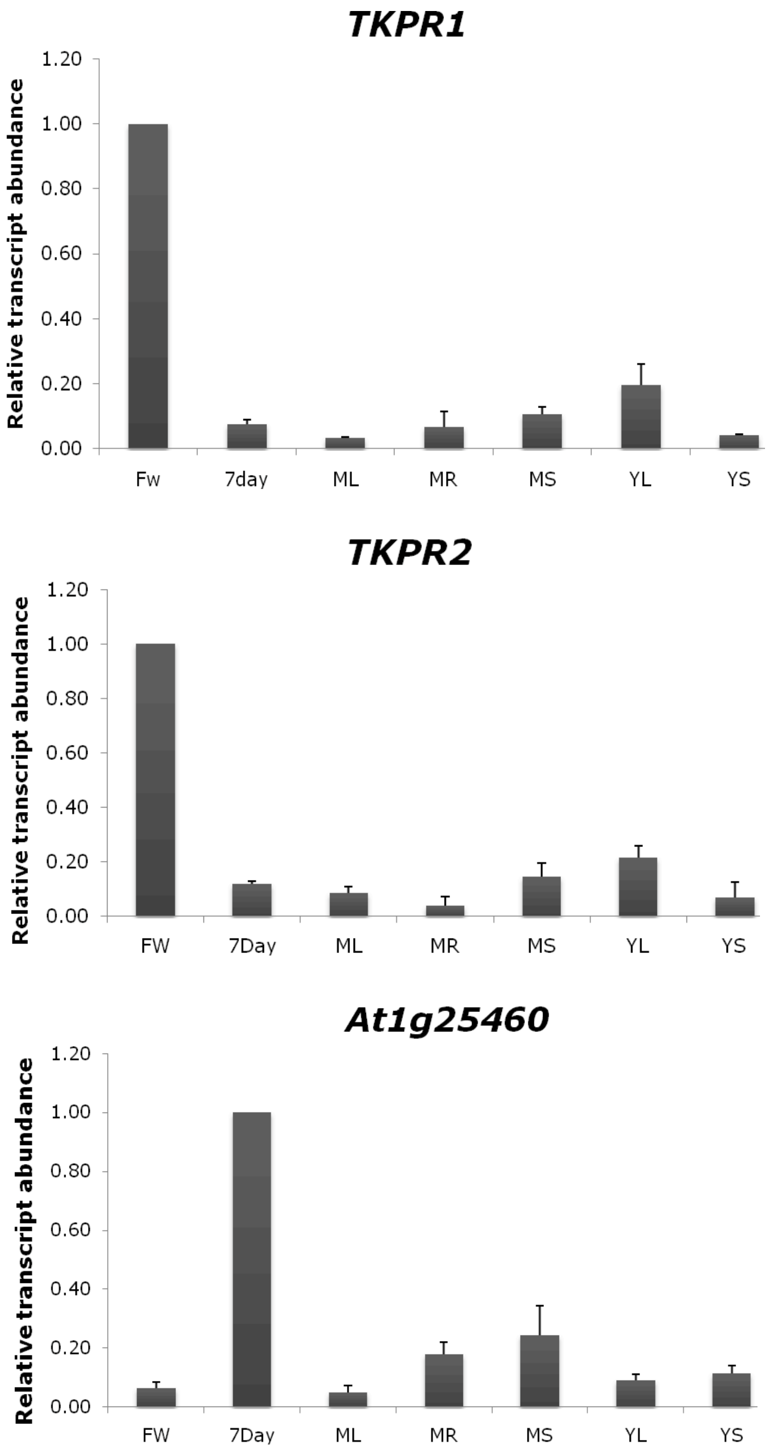
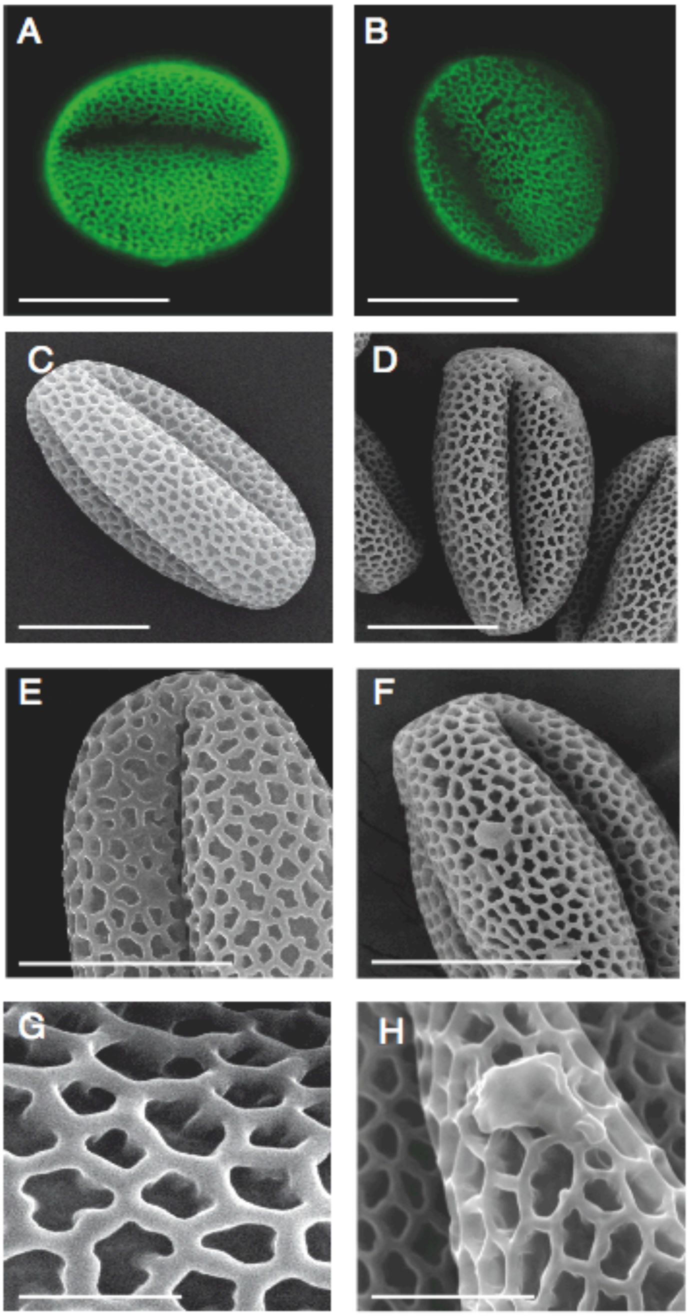


Supplemental Figure 1. Multiple alignment of oxido-reductase predicted protein sequences obtained with ClustalW.

Black boxes indicate identical amino acids and grey boxes similar residues. The putative N-terminal NAD(P)H binding domain is underlined and amino acids strictly conserved in the N-terminal sequence are indicated by an asterisk. Accession numbers are : At1g15950 for *Arabidopsis* cinnamoyl-CoA reductase (AtCCR1) ; At5g42800 for *Arabidopsis* dihydroflavonol reductase (AtDFR) ; AAH92571 for rat 3-β hydroxysteroid deshydrogenase/isomerase ; BAI54225 for *E. coli* UDP-galactose-4-epimerase.

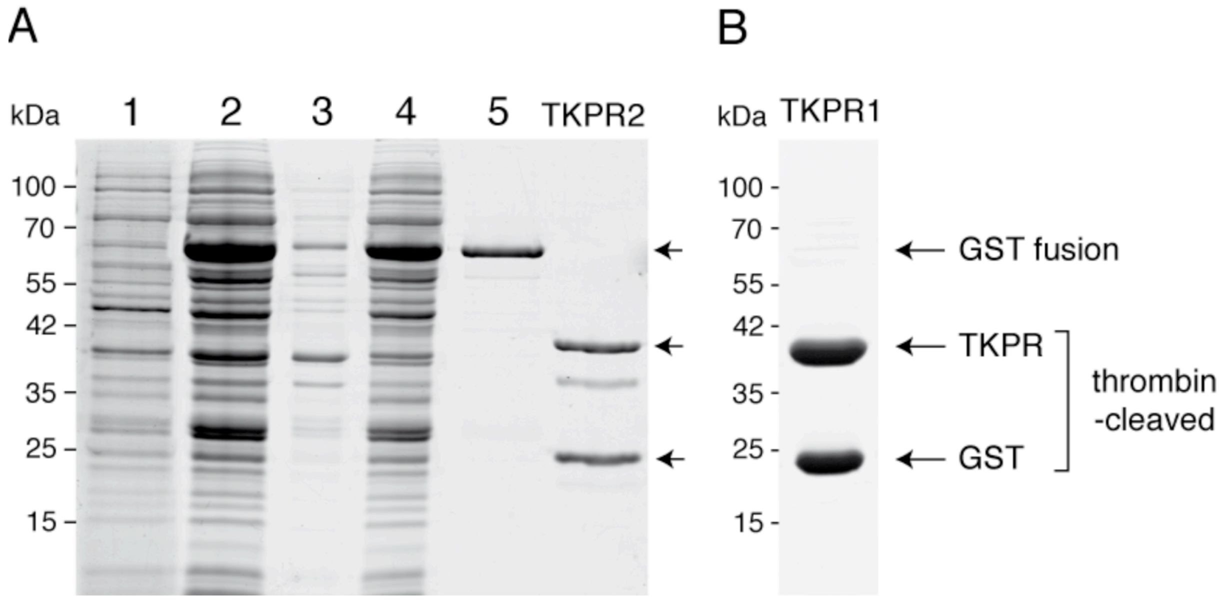


Supplemental Figure 2. Developmental expression profiles of *TKPR1, TKPR2* and *At1g25460.*
Relative *TKPR1, TKPR2* and *At1g25460* expression levels in various *Arabidopsis* organs were analyzed by quantitative RT-PCR. Expression was calculated using the ΔΔCT method and is represented relative to the organ with the highest level of expression (flower buds for *TKPR1* and *TKPR2*; 7-day old seedlings for *At1g25460*) set at 1.0. *Actin2* (*At3g18780*) was used as a reference gene. Primer sequences are given in Supplemental Table 2 online. Bars represent standard deviations from the means of triplicate determinations. 7day, 7-day old seedlings; Fw, flower; ML, mature leaf; MR, mature root; MS, mature stem; YL, young leaf; YS, young stem.

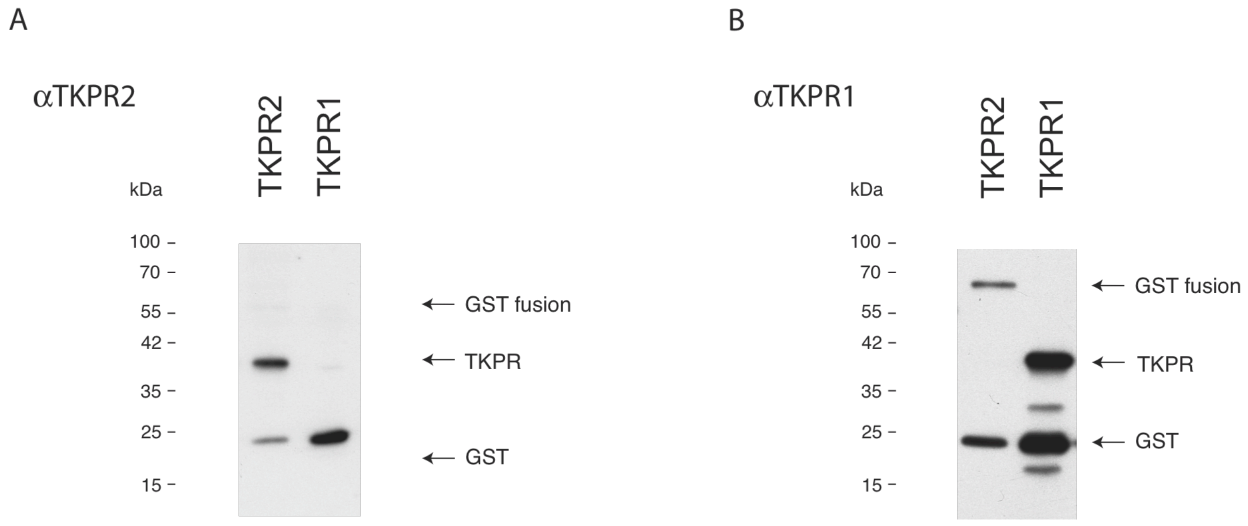


Supplemental Figure 3. Comparison of exine architecture in wild-type and *tkpr2-2* pollen.

(A), (C), (E) and **(G)** Wild-type pollen
(B), (D), (F) and **(H) :** *tkpr2-2* pollen
(A) and **(B)** Epifluorescence microscope images of wild-type and mutant pollen. Pollen was stained with the fluorescent dye auramine O and visualized using fluorescein isothiocyanate settings.
(C) to **(H)** Scanning electron micrographs of wild-type and mutant pollen grains.
Scale bars = 10 µm (**A-F**), 2 µm (**G, H**)



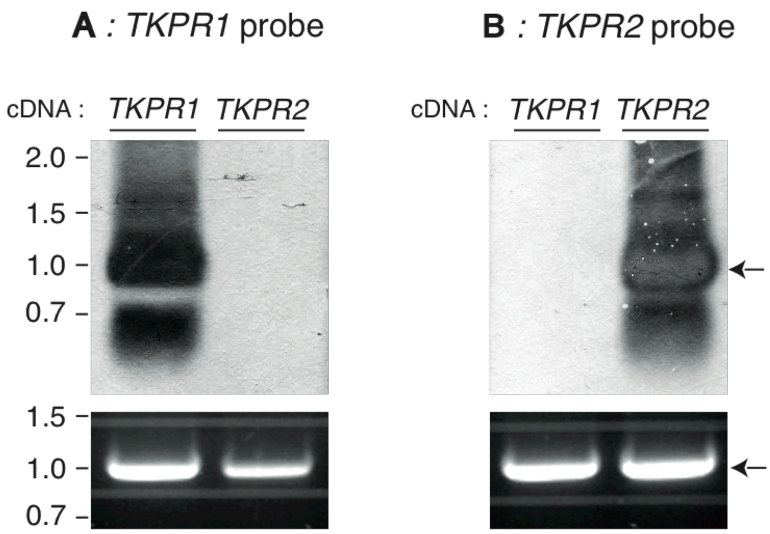
Supplemental Figure 4. Analysis of recombinant protein preparations at different steps of purification.
Bacterial protein extracts were prepared and purified as described in Methods section. Protein preparations were analyzed by electrophoresis on SDS-polyacrylamide gels and Coomassie Blue staining.
(A) Purification steps of TKPR2 are illustrated. Lane 1, total protein from uninduced bacteria ; lane 2, total protein from induced bacteria that was further fractionated; lane 3, soluble fraction ; lane 4, insoluble fraction ; lane 5, fusion protein from soluble fraction purified by affinity chromatography on glutathion-agarose ; TKPR2, thrombin-cleaved preparation containing reductase and GST proteins (arrows).
(B) Purified TKPR1 preparation after affinity chromatography and thrombin cleavage.



Supplemental Figure 5. Characterization of antibodies raised against TKPR proteins. Purified recombinant TKPR1 and TKPR2 proteins were immunoblotted with each polyclonal serum.

(A) Polyclonal antibodies raised against TKPR2 recombinant protein recognized TKPR2 but not TKPR1. The presence of anti GST antibodies was also evidenced.

(B) Antibodies raised against TKPR1 were specific for TKPR1 protein and did not react with TKPR2. Anti GST antibodies were also present in the serum and detected GST protein.



Supplemental Figure 6. Specificity of *TKPR* nucleotidic probes.
Specificity of probes used for *in situ* hybridization was evaluated by Southern blotting. Coding sequences of TKPR transcripts were amplified by PCR using gene-specific primers. Amplicons of 0.95 kb predicted size were visualized on EtBr-stained 0.7% agarose gels (lower panels) before transfer onto nylon membranes. Blots were hybridized separately with digoxigenin-labelled riboprobes corresponding to coding sequences of *TKPR1* **(A)** or *TKPR2* **(B)**. An indication of size positions in kb is given on the left. Arrows indicate positions of amplicons on gel and blot. Data show the absence of cross-hybridization between the two transcripts.

Supplemental Table 1. Primers used in cloning, genotyping and RT-PCR experiments.

Gene (mutant line)	Type of study	Forward Primer (5' → 3')	Reverse Primer (5' → 3')
AT4g35420	Quantitative RT-PCR	CAGAGATCCAGGAAATGAGAAGAAAC	AAGCACCGGAGAAGCAGTATGGAA
ACTIN2		CCAGAAGGATGCATATGTTGGTGA	GAGGAGCCTCGGTAAGAAGA
AT4g35420 (SAIL_837_D01)	Genotyping	GATGCCAAGGAGTGTTCCAT ATTTTGCCGATTTCGGAAC	TGGACCCAAAAACGAGTCAT TGGACCCAAAAACGAGTCAT
AT4g35420 (FLAG019D03)		GATGCCAAGGAGTGTTCCAT GATGCCAAGGAGTGTTCCAT	CTCGAGCTCTTATGGAAGAACAGTAGATAA CGTGTGCCAGGTGCCCACGGAATAGT
AT1G68540 (SALK_129453C)		GTGCCAAGTCTAAAGCCACA ATTTTGCCGATTTCGGAAC	TGAAGGATCCAAATCCCAAC TGAAGGATCCAAATCCCAAC
AT1G68540 (GK838H09)		AAACACATGCGAAGACATGG ATATTGACCATCATACTCATTGC	CCATCAACACCATTCACTGC CCATCAACACCATTCACTGC
AT4g35420	cDNA cloning for protein production	GAGGAATTCCAATGGATCAAGCAAAGGGAAA EcoRI ATG	CTCGAGCTCTTATGGAAGAACAGTAGATAA SacI Stop
AT1G68540		GAGTCTAGAGATGTCTGAGTATTTGGTAACG XBaI ATG	CTCCCATGGTTAGAGCAGACCCTTCTTCTG PstI Stop
AT4g35420	In situ hybridiza-tion	GATCCAGGAAATGAGAAGAAAC	GTTTCTCAAACCTCTTGGGG
		CATAATACGACTCACTATAGGGTTTCTCAAACCTCT TGGGG	CATAATACGACTCACTATAGGGATCCAGG AAATGAGAAGAAAC
AT1g68540		CATAATACGACTCACTATAGGATGTCTGAGTATTTG GTAACTGG	CATAATACGACTCACTATAGGTTAGAGCA GACCCTTCTTCTGAAAAC
		TTA GAG CAG ACC CTT CTT CTG AAA AC	ATG TCT GAG TAT TTG GTA ACT GG
AT4g35420	RT-PCR	GAGGAATTCCAATGGATCAAGCAAAGGGAAA	CTCGAGCTCTTATGGAAGAACAGTAGATAA
AT1G68540		GAGTCTAGAGATGTCTGAGTATTTGGTAACTG	CTCCCATGGTTAGAGCAGACCCTTCTTCTG
TUBULIN3		GTGGAGCCTTACAACGCTACTT	GACAGCAAGTCACACCAGACAT
AT4g35420	GFP fusion	GGGGACAAGTTTGTACAAAAAAGCAGGCT*TCATG*G ATCAAGCAAAGGGAAA	*GGGGACCACTTTGTACAAGAAAGCTGGGT CTGGAAGAACAGTAGATAAA*
AT1g68540		GGGGACAAGTTTGTACAAAAAAGCAGGCT*TCATG*T CTGAGTATTTGGTAAC	*GGGGACCACTTTGTACAAGAAAGCTGGGT C*GAGCAGACCCTTCTTCTGA

Supplemental Table 2. Genes and expression data used for constructing the phylogenetic tree of Figure 10.

Gene name	Species	Gene model or GenBank ID	Expression	Reference
AtTKPR2	***Arabidopsis thaliana***	**At1g68540**	**tapetum**	**this study**
AtTKPR1	***Arabidopsis thaliana***	**At4g35420**	**tapetum**	**this study, Tang et al., 2008**
OsDFR2	*Oryza sativa*	Os09g32020	tapetum	Yau et al., 2005
At1g25460	*Arabidopsis thaliana*	At1g25460	young seed, silique	eFP Browser[3]
O. sativa	*Oryza sativa*	Os01g03670	tapetum	Huang et al., 2009
T. aestivum	*Triticum aestivum*	TC323911	flower bud	DFCI EST[1]
L. solanum 2	*Lycopersicon solanum*	BI929118.1	flower bud	ncbi EST[2]
L. solanum 1	*Lycopersicon solanum*	BI930405.1	flower bud	DFCI EST[1]
B. rapa 1	*Brassica rapa*	EX048625.1	flower bud	ncbi EST[2]
B. rapa 2	*Brassica rapa*	EX049142.1	anther	ncbi EST[2]
R. communis 1	*Ricinus communis*	EE255951.1	flower	ncbi EST[2]
P. trichocarpa 1	*Populus trichocarpa*	EEE88913.1	male catkins	eFP Browser[3]
P. patens 1	*Physcomitrella patens*	EDQ63261.1	green sporophyte	ncbi EST[2]
P. patens 2	*Physcomitrella patens*	EDQ65809.1	green sporophyte	ncbi EST[2]
V. vinifera 1	*vitis vinifera*	CBI37707.1	flower bud	ncbi EST[2]
P. trichocarpa 2	*Populus trichocarpa*	BU878087	xylem, root	eFP Browser[3]
V. vinifera 2	*vitis vinifera*	CBI27024.1	nd	
Z. mays	*Zea mays*	ACG40388.1	nd	
R. communis 2	*Ricinus communis*	EEF30759.1	nd	
PtANR2	*Populus trichocarpa*	EEE97882.1		
PtANR1	*Populus trichocarpa*	EEE86150.1		
AtANR/BAN	*Arabidopsis thaliana*	At1G61720		
Os04g53920	*Oryza sativa*	*Os04g53920*		
Os04g53850	*Oryza sativa*	*Os04g53850*		
PtDFR1	*Populus trichocarpa*	EEE80032.1		
AtDFR	*Arabidopsis thaliana*	At5G42800		
VvDFRL	*vitis vinifera*	CAA53578.1		
ZmDFRL	*Zea mays*	AF347696_1		
OsDFRL	*Oryza sativa*	AAB58474.1		
PtCCR2	*Populus trichocarpa*	EEE70443.1		
AtCCR1	*Arabidopsis thaliana*	At1G15950		
ZmCCRL	*Zea mays*	ACN31052.1		
OsCCRL	*Oryza sativa*	Os08g04415		

[1] http://compbio.dfci.harvard.edu/tgi/plant.html
[2] http://blast.ncbi.nlm.nih.gov/Blast.cgi
[3] http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi
nd, no data

Supplemental Reference

Huang, M. D., Wei, F. J., Wu, C. C., Hsing, Y. I. and Huang, A. H. (2009) Analyses of advanced rice anther transcriptomes reveal global tapetum secretory functions and potential proteins for lipid exine formation. Plant Physiol **149:** 694-707.