

Supplemental Material

for *Plant Physiology* article

Analyses of advanced rice anther transcriptomes reveal global tapetum secretory functions and potential proteins for lipid exine formation

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Table S1-Early-Vacuolated

TIGR_gene	Annotation	SBS							MPSS										Insertion mutant	Activation mutant
		Developing anthers						Pollen	Germina seed	Young leaf	Young root	Mature leaf	Mature root	Stem	Immature panicle	Ovary & stigma	Pollen			
		Meiosis	Tetrad	Early vacu	Late vacu	Mitosis	Mature pollen													
Os03g59380	Lipid-transfer protein	1,358	17,792	20,728	35,555	512	169	1	0	0	0	0	0	0	0	399	29	3		TRIM
Os01g41710	Chlorophyll a-b binding protein	1,373	11,472	17,209	17,289	2,617	45	0	29	20	0	0	0	0	0	369	55	0	PFG, TRIM	
Os09g35700	Lipid-transfer protein	16,153	49,751	13,603	81	1	4	1	0	0	0	0	0	0	0	708	0	0		
Os01g41170	Unknown protein	0	1,914	8,675	22,039	1,105	7	0	0	0	0	0	0	0	0	318	0	242	PFG	
Os07g37850	Unknown protein	143	23	7,997	31	0	0	0	0	0	0	0	0	0	23	1,410	10	0		TRIM
Os08g45150	Proline-rich protein	6	1,413	5,527	2,515	1	1	3	0	0	0	0	0	0	0	256	0	0	RMD, UCD	
Os02g45530	Glucose-methanol-choline oxidoreductase	131	7,496	3,672	4	0	0	0	0	0	0	0	0	0	0	0	0	0	TRIM	TRIM
Os05g40010	Lipid-transfer protein	5	381	3,165	2,495	15	90	0	0	0	0	0	5	0	0	18	17	CSIRO		
Os02g01980	Proline-rich protein	1	131	2,831	0	0	0	4	0	0	0	0	0	0	96	0	0			
Os10g42620	Dihydroflavonol-4-reductase	9	4,613	2,445	345	7	1	1	22	0	0	0	0	8	26	3	0	PFG, RMD, TRIM		TRIM
Os07g46210	Unknown protein	2	5,127	2,210	26	0	0	0	0	0	0	0	0	0	25	0	0			TRIM
Os04g33930	Lipid binding protein	0	0	2,114	38	1	0	0	0	0	0	0	0	0	0	0	0	0	PFG	TRIM
Os02g09420	Cyclase	116	5	2,015	2	0	0	0	0	0	0	0	0	0	0	15	0			
Os01g09320	NADP-dependent malic enzyme	909	876	1,960	527	85	44	43	0	24	42	0	54	38	25	105	0			
Os02g01190	Unknown protein	80	146	1,747	0	0	0	0	0	0	0	0	0	0	508	0	0	RMD, TRIM		
Os01g37950	Unknown protein	392	789	1,700	4,045	214	797	4	19	0	0	0	2	36	22	0	0			
Os07g37100	Nucleoside transporter	28	275	1,650	316	24	30	44	96	110	0	10	21	0	0	4	3	PFG, RTIM, TRIM		TRIM
Os01g03670	Dihydroflavonol-4-reductase	47	11,738	1,649	16	5	3	1	0	0	0	0	0	0	117	0	0			
Os08g41950	SRF-type transcription factor	1,720	119	1,621	67	26	28	13	0	0	0	0	0	0	17	297	10	PFG, TRIM, UCD		TRIM
Os08g03520	Glycine-rich protein 2b	1,782	2,108	1,598	3,136	206	66	2	47	15	0	0	72	87	69	167	0			
Os12g18960	Nodulin-like protein	240	426	1,584	2,987	763	439	2	0	0	0	0	0	0	0	0	0	22	PFG	
Os03g10320	Unknown protein	6	0	1,332	2	0	0	0	0	4	0	0	0	0	0	0	0	6	PFG	TRIM
Os05g35740	Subtilisin propeptide domain containing protein	73	35	1,318	87	27	16	1	58	54	0	0	0	0	2	0	0	PFG		
Os08g43290	Lipid-transfer protein	554	6,768	1,261	28	0	0	0	0	0	0	0	0	0	3,537	0	0			TRIM
Os02g30200	PE-PGRS family protein	5	296	1,241	1,775	49	8	2	0	0	0	0	0	0	0	0	0	0	PFG, UCD	TRIM
Os03g39610	Chlorophyll a-b binding protein	569	1,070	1,205	716	248	4	0	0	0	0	0	0	0	346	97	0	PFG		
Os03g08790	Aspartic proteinase	61	158	1,128	1	0	0	0	33	0	0	0	4	137	123	12	0	Genoplante		TRIM
Os02g49870	Unknown protein	194	2	1,127	4	0	0	0	0	9	0	0	0	117	0	9	0			TRIM
Os10g37420	Cytochrome b5	246	648	951	587	70	70	0	0	6	0	0	0	0	0	0	0	GSNU		TRIM
Os05g07700	60S ribosomal protein L10-3	555	1,323	924	1,716	411	117	6	5	20	0	1	14	0	10	0	0	PFG, TRIM		TRIM
Os03g47910	Unknown protein	807	2,357	923	3	0	0	0	0	0	0	0	0	0	489	0	0			
Os08g03450	60S ribosomal protein L37	751	1,562	912	1,887	411	72	7	10	9	0	0	2	0	0	11	0	RMD		
Os09g35800	UDP-glucose 4-epimerase	28	103	895	1,229	10	6	4	0	19	0	0	0	0	0	0	0	TRIM		TRIM
Os08g29770	Endoglucanase	0	402	876	1,017	164	63	0	0	16	0	0	0	0	0	0	0	PFG		
Os06g12320	Amino acid/polyamine transporter	1	140	873	543	11	0	0	0	20	0	0	0	0	0	0	0	PFG		
Os06g12350	Amino acid transporter	0	4	870	84	3	1	0	0	0	0	0	0	0	0	0	0	PFG		
Os02g07820	Unknown protein	12	270	831	2,719	245	71	0	0	0	0	0	0	0	0	0	0	6		
Os03g15710	Strictosidine synthase	33	942	830	43	5	1	0	0	0	0	0	0	0	22	0	0			
Os08g03682	Cytochrome P450	43	1,705	812	5	1	0	2	0	0	0	0	0	3	469	0	0			
Os01g56530	LOB domain protein	0	849	808	62	1	1	0	0	0	0	0	0	0	0	0	0	TRIM		
Os03g18420	NADH-ubiquinone oxidoreductase	386	98	786	48	114	38	35	38	0	0	0	0	17	92	30	56	PFG		
Os01g05670	3-oxo-5-alpha-steroid 4-dehydrogenase	275	1,217	772	398	72	23	1	0	0	0	0	1	0	29	21	0	PFG, RMD, RTIM		TRIM
Os05g30580	Subtilisin-like protease	0	2	748	1	0	0	0	0	0	0	0	0	0	0	0	0	PFG		TRIM
Os10g25230	ZIM motif family protein	453	425	743	2,315	146	42	13	0	0	0	0	0	8	0	14	0			TRIM
Os06g40150	Ethylene responsive element	11	67	733	72	1	0	0	0	0	0	0	0	0	6	0	0	PFG, TRIM		TRIM
Os01g53240	RD22-like protein	108	10	732	108	0	0	1	16	0	0	0	0	21	366	0	0			
Os06g42310	β-galactosidase	0	1	732	5	0	0	40	0	0	0	0	0	0	0	0	0			
Os12g37870	ATP-citrate synthase	380	259	726	601	106	43	27	0	0	10	0	0	0	0	0	0	PFG, TRIM		TRIM
Os11g09280	Protein disulfide isomerase	921	182	707	215	13	8	0	0	0	0	5	0	7	12	55	0	PFG		
Os11g41610	40S ribosomal protein S29	791	916	704	2,387	641	87	4	0	14	0	0	2	0	0	19	0	RMD, ZJU		

Table S2. *Anther-minus-pollen transcripts whose ortholog transcripts/proteins are known to be present in or restricted to the tapeta in anthers of different species.* Anther-minus-pollen transcripts are those that possess TPM >5X those in pollen. Their presence in various anther, pollen (current SBS results) and other organs (reported MPSS results) transcriptomes are shown. Rice ortholog transcripts with TPM lower than 20 are excluded to avoid background noises.

Transcripts of rice genes	Transcripts/proteins of rice orthologs known to be restricted to the tapetum in anthers	SBS							MPSS									
		Developing anthers						Pollen	Germina seed	Young leaf	Young root	Mature leaf	Mature root	Stem	Immature panicle	Ovary & stigma	Pollen	
		Meio	Tetrad	Early vacu	Late Vacu	Mitosis	Mature pollen											
TPM																		
Os01g49650	TomA5B (Tomato) ¹	2,632	1,850	39	9	0	0	0	0	0	0	0	0	0	0	1,591	0	0
Os03g18850	LLA-15 (Lily) ²	24	2	1	2	0	0	1	0	0	13	0	3	0	0	25	0	0
Os03g59380	bvLTP-1 (Sugar beet) ³	1,358	17,792	20,728	35,555	512	169	1	0	0	0	0	0	0	0	399	29	3
Os03g07140	MS2 (Arabidopsis) ⁴	2	40	624	1	2	0	1	0	0	0	0	0	0	0	274	0	0
Os04g39470	AtMYB103 (Arabidopsis) ⁵	5	59	1	0	0	0	0	0	2	0	0	0	0	2	0	0	0
Os07g22850	PrChS1 (<i>Pinus radiata</i>) ⁶	92	12	68	0	0	0	0	0	0	0	0	0	0	99	0	0	0
Os07g37090	RA39 (Rice) ⁷	0	143	293	0	0	0	0	0	0	0	0	0	0	174	0	0	0
Os08g03682	CYP703 (Arabidopsis) ⁸	43	1,705	812	5	1	0	2	0	0	0	0	0	3	469	0	0	0
Os08g38810	RAFTIN (Rice) ⁹	35	25	136	47	0	0	0	0	0	0	0	0	0	609	0	0	0
Os08g43290	Osc4 (Rice) ¹⁰	554	6,768	1,261	28	0	0	0	0	0	0	0	0	0	3,537	0	0	0
Os09g27620	MS1 (Arabidopsis) ¹¹	1	356	53	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Os09g35700	YY1 (Rice) ¹²	16,153	49,751	13,603	81	1	4	1	0	0	0	0	0	0	708	0	0	0
	Toma108z (Tomato) ¹³																	
	Mzm3-3 (Maize) ¹⁴																	
Os10g34360	YY2 (Rice) ¹²	5	134	78	0	0	0	0	0	0	0	0	0	0	1,333	0	0	0
	PrChS1 (<i>Pinus radiata</i>) ⁶																	
Os01g68870	MSP1 (Rice) ¹⁵	25	32	12	9	3	1	0	0	0	0	0	0	24	3	40	0	0
	EMS/EXS (Arabidopsis) ¹⁶																	
Os02g02820	TDR (Rice) ¹⁷	97	3	110	1	0	0	0	0	0	0	0	0	0	110	0	0	0
	DYT1 (Arabidopsis) ¹⁸																	
Os02g53200	Tag1 (Tobacco) ¹⁹	30	1	1	0	0	0	0	227	24	22	4	78	253	386	224	1	0
Os04g38480	SERK1 (Arabidopsis) ²⁰	22	13	14	15	15	6	1	0	0	0	0	24	0	0	0	0	0
Os04g57490	OsCPI1 (Rice) ²¹	0	1	44	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Os06g06980	AtTSM1 (Arabidopsis) ²²	70	1	9	1	0	1	0	519	305	210	269	295	1,323	1,367	259	0	0
Os09g32550	A6 (Arabidopsis) ²³	59	24	7	18	7	3	0	0	0	0	0	0	0	25	77	0	0
Os09g30320	RA8 (Rice) ²⁴	6	152	283	166	4	0	0	0	0	0	0	0	0	636	2	213	0
Os09g36730	AtMYB32 (Arabidopsis) ²⁵	11	148	139	19	0	0	0	190	29	471	2	204	68	361	0	0	0
Os12g02310	PrLTP1 (<i>Pinus radiata</i>) ⁶	23	0	8	0	0	4	0	704	316	0	0	0	605	206	0	0	0

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Table S3. *Anther-minus-other-organs transcripts with TPM ≥100 that encode secretory proteins.*

Transcripts with TPM ≥1,000 are shaded.

TIGR_gene	Annotation	SBS						MPSS								
		Developing anthers					Pollen	Germina	Young	Young	Mature	Mature	Stem	Immature	Ovary &	Pollen
		Meiosis	Tetrad	Early vacu	Late Vacu	Mitosis	Mature pollen	seed	leaf	root	leaf	root	panicle	stigma		
		TPM														
Os01g11710	Esterase	67	146	62	31	5	7	0	0	0	0	0	0	0	0	0
Os01g12020	Lipid-transfer protein	9,549	9,683	105	29	19	8	0	0	0	0	0	0	120	0	
Os01g13200	Unknown protein	204	5	210	2	1	0	15	0	5	0	0	14	0	23	
Os01g14880	Hypothetical protein	0	813	361	2	0	0	0	0	0	0	0	0	125	0	
Os01g14940	Pectinesterase inhibitor	0	0	0	8	4,562	2,223	183	0	0	0	0	0	0	33	
Os01g15610	Unknown protein	25	40	27	146	3,619	250	9	0	0	0	1	0	0	0	
Os01g15830	Peroxidase	408	1	175	0	0	0	0	0	0	0	1	0	0	0	
Os01g15970	Unknown protein	82	179	4	4	0	0	8	8	5	0	0	2	0	0	
Os01g22380	Unknown protein	376	3	7	0	0	0	0	0	0	0	0	0	39	0	
Os01g33670	Unknown protein	0	15	93	233	0	52	0	0	0	0	0	0	7	0	
Os01g34920	β-galactosidase	0	6	252	37	0	0	0	0	0	0	0	0	0	24	
Os01g49650	Lipid-transfer protein	2,632	1,850	39	9	0	0	0	0	0	0	0	1,591	0	0	
Os01g53240	RD22-like protein	108	10	732	108	0	0	1	16	0	0	0	21	366	0	
Os01g66700	β-hexosaminidase	730	96	43	4	8	10	2	0	0	0	0	98	0	12	
Os01g68589	Lipid-transfer protein	129	0	41	0	0	0	0	4	0	8	0	0	16	0	
Os02g01190	Unknown protein	80	146	1,747	0	0	0	0	0	0	0	0	508	0	0	
Os02g10520	Protease	2	90	626	19	0	0	0	0	0	0	0	7	0	0	
Os02g12270	Hypothetical protein	0	0	20	261	16	1	0	0	0	0	0	0	0	0	
Os02g22480	Glycosyltransferase	98	129	103	106	45	36	0	0	0	0	0	0	13	16	
Os02g28970	Unknown protein	247	41	2	0	0	0	0	0	0	0	0	0	0	0	
Os02g40440	Proline-rich protein	74	2	291	2	0	0	0	0	0	0	0	31	3	0	
Os02g42310	Serine carboxypeptidase	11	0	285	6	0	0	0	15	0	0	0	16	6	0	
Os02g45530	GMC oxidoreductase	131	7,496	3,672	4	0	0	0	0	0	0	0	0	0	0	
Os02g48110	Heat shock protein	254	293	12	136	90	10	5	10	0	0	19	5	20	0	
Os03g08790	Aspartic proteinase	61	158	1,128	1	0	0	0	33	0	0	0	4	137	123	
Os03g24300	Hypothetical protein	918	77	16	0	0	0	0	0	0	0	0	263	0	0	
Os03g24910	Unknown protein	181	125	2	20	1	0	0	14	0	0	0	1	0	4	
Os03g26430	Aldose 1-epimerase	6	0	272	0	0	1	0	0	0	0	0	0	0	0	
Os03g43790	Unknown protein	7	241	3	0	0	0	0	0	0	0	0	0	0	0	
Os03g47910	Unknown protein	807	2,357	923	3	0	0	0	0	0	0	0	0	489	0	
Os03g48200	Ribosome inactivating protein	106	28	0	0	0	0	0	0	0	0	0	0	110	0	
Os03g48220	Unknown protein	125	179	177	4	0	0	0	0	0	0	0	173	20	0	
Os03g49600	β-glucosidase	1,010	635	171	73	24	39	5	0	0	0	0	34	25	0	
Os03g52160	Unknown protein	280	43	1	0	0	0	0	0	0	0	0	34	0	0	
Os03g53310	Suppressor/enhancer of lin-12	161	271	96	203	201	95	51	0	3	0	0	0	33	1	
Os03g60370	Acid phosphatase	114	6	97	9	2	2	4	0	0	0	2	0	2	3	
Os03g63390	Chemocyanin	3	233	31	3	0	0	0	0	0	0	0	0	0	0	
Os04g33930	Lipid binding protein	0	0	2,114	38	1	0	0	0	0	0	0	0	0	0	
Os04g35140	Protease	1	72	172	2	1	6	0	0	0	0	0	0	65	0	
Os04g39590	Arabinogalactan protein	0	0	2	26	284	44	5	0	0	0	0	0	0	21	
Os04g48400	GMC oxidoreductase	135	603	141	2	0	0	0	0	0	0	0	0	551	0	
Os04g55660	GDSL-like lipase	0	26	337	919	25	1	3	0	0	0	0	0	0	10	
Os04g58120	Unknown protein	4,960	243	28	0	0	0	0	0	0	0	0	0	165	0	
Os05g05920	Unknown protein	0	300	48	1	0	0	0	0	0	0	0	0	165	0	
Os05g10210	Glycoprotein	131	14	2	0	0	0	0	0	0	0	0	25	190	0	
Os05g30400	Unknown protein	1,555	1	59	1	0	0	0	0	0	0	0	0	43	0	
Os05g35740	Subtilisin propeptide domain	73	35	1,318	87	27	16	1	58	54	0	0	0	2	0	
Os05g40010	Lipid-transfer protein	5	381	3,165	2,495	15	90	0	0	0	0	5	0	18	17	
Os05g44120	Unknown protein	1,750	1,003	164	8	1	5	0	0	0	0	0	0	448	0	
Os05g44760	Hexokinase	20	55	56	137	524	236	26	0	9	0	1	9	0	32	
Os06g05740	Unknown protein	276	8	31	7	4	0	3	0	28	0	0	1	0	0	
Os06g42310	β-galactosidase	0	1	732	5	0	0	40	0	0	0	0	0	0	0	
Os06g44660	Arabinogalactan protein	0	114	14	557	1,196	21	16	0	0	0	0	0	12	54	
Os06g48020	Peroxidase	8	36	64	224	72	44	0	11	0	0	0	0	0	11	
Os07g23730	Thaumatin-like protein	11	8	27	154	37	1	1	0	0	0	0	0	0	2	
Os07g37850	Unknown protein	143	23	7,997	31	0	0	0	0	0	0	0	23	1,410	10	
Os07g40740	Heparanase-like protein	0	5	102	947	42	0	1	0	0	0	0	0	0	0	
Os07g41650	Pectinesterase	3	196	90	213	10	4	0	0	0	0	0	0	111	34	
Os07g46210	Aspartic acid-rich protein	2	5,127	2,210	26	0	0	0	0	0	0	0	0	25	0	
Os08g02094	Proline-rich protein	332	129	56	83	39	1	0	0	0	0	0	0	0	0	
Os08g10670	Aspartyl protease	223	1	3	0	0	0	0	18	0	0	0	23	149	0	
Os08g27210	Unknown protein	0	118	13	0	0	0	0	0	0	0	0	0	0	0	
Os08g29770	Endoglucanase	0	402	876	1,017	164	63	0	16	0	0	0	0	0	0	
Os08g43290	Lipid-transfer protein	554	6,768	1,261	28	0	0	0	0	0	0	0	0	3,537	0	
Os08g45150	Proline-rich protein	6	1,413	5,527	2,515	1	1	3	0	0	0	0	0	256	0	
Os09g13930	Unknown protein	14	655	6	1	1	2	0	0	0	0	0	0	396	0	
Os09g19930	GMC oxidoreductase	124	75	42	58	2	0	0	0	0	0	1	89	0	0	
Os09g30478	Unknown protein	43	102	78	140	105	113	19	0	0	0	0	0	0	0	
Os09g35700	Lipid-transfer protein	16,153	49,751	13,603	81	1	4	1	0	0	0	0	0	708	0	
Os10g05720	Lipid binding protein	0	79	131	1,325	0	0	0	0	0	0	0	0	71	0	
Os10g36760	Peptidase	12	56	46	103	23	5	1	0	10	0	2	0	0	0	
Os11g33134	Unknown protein	0	1	1	23	182	9	1	0	0	0	0	0	0	16	
Os12g15470	Peptidase	10	33	96	178	13	23	0	0	6	0	1	0	0	0	

Table S4. Characteristics of rice spikelets and anthers of progressive developmental stages used for the construction of SBS transcriptomes.

Developing anthers (stages)

		Meiosis	Tetrad	Early vacu	Late vacu	Mitosis	Mature Pollen
spikelet	Length (mm)	2.7±0.3	3.5±0.3	4.9±0.3	5.9±0.2	6.0±0.4	6.1±1.4
	Width (mm)	1.5±0.1	1.7±0.1	2.0±0.1	3.0±0.1	3.3±0.2	3.1±0.3
	Bracket Color	White	Yellowish white	Yellowish white	Light green	Green	Green
anther	Length	0.6±0.1	0.9±0.1	1.1±0.1	1.4±0.1	1.8±0.2	2.1±0.1
	Ratio*	0.29	0.34	0.41	0.42	0.53	0.66
	Color	White	Yellowish white	Yellowish white	Light yellow	Yellow	Yellow

Duration between stages (d)**

	1.2	0.84-1.23	1.59-1.85	2.67-3.25	~2
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*, ratio of the longitudinal lengths of anther and spikelet.

** Durations between stages are averaged values from several collections and depend on the weather and positions of the spikelets on the plants.

Table S5. *Primers used for RT-PCR analysis.*

Gene ID	Annotation	Direction	Primer
Os09g35700	Lipid transfer protein	F	GATGGAGATGGTGTGCCAAGG
		R	TGAACGATCAGCAGAGCAAGC
Os08g44840	Acyltransferase	F	TTCGACGGGATGGTCTACCTC
		R	TAGCAAGCAACAAGGAATCCATG
Os04g48210	Cytochrome P450	F	GCTCATCTCCTTCCCGCTGT
		R	TCGTTTCATGCTCCACCGTG
Os05g40010	Lipid transfer protein	F	GTTGCACACAGGAGTTTGAGGT
		R	TTGGTGATTGATCGGACAAGC
Os02g07820	Unknown protein	F	GATGAGCTTTCTTGAGCAGGC
		R	TGACGCAAGTGTAGCTCTCAGAG
Os11g11710	Unknown protein	F	CTCTGCAACACCTGCTGCC
		R	AGGTCTTATCGGCGGTTGATC
Os10g40090	β -expansin	F	ATGATCTCCTGCGGCAACG
		R	GAGCCCTTCTCGACGTGGA
Os05g46530	Pectinesterase inhibitor	F	TGAAGCTGTTCGATGAGGAAGG
		R	CGAGATACGCCACCACTTCTG
Os07g26480	Desiccation-associated protein	F	AGCGGCAGCATGGACATCA
		R	TTGGACTTCTTGGGCACGG
Os08g03682	Cytochrome P450	F	CGATCATGGGCTACGACATCC
		R	AGTCGAAGCAGTGGAAGAGCC
Os01g26000	Acyltransferases	F	GCACAGCTTACTGTTGGAGTACGG
		R	GGTTGAGGAAGTCGAAGAAGTCG
Os01g12020	Lipid transfer protein	F	CTCCCCTCCTCCACTAGACCAC
		R	ACTCGATTCTCAGCTCTCAG
Os01g46950	Lipid transfer protein	F	TCCAGCAAGAGATCCATCAAGC
		R	TCTGCCTCACTGAACAATGGTC
Os08g43290	Lipid transfer protein	F	CACACACTCCCATCACTCCA
		R	TGTTGAAATCCCTCCTTTGG
Os11g37280	Lipid transfer protein	F	GTGCCGCCTCTCTACTCTTGC
		R	CATAGCACGACAGTAGCACGAGC
Os03g50890	Actin1	F	CCTCTTCCAGCCTTCCTTCAT
		R	ACGGCGATAACAGCTCCTCTT