Genome-Wide Gene Expression Profiling Reveals Conserved and Novel Molecular Functions of the Stigma in Rice[1][W]

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In angiosperms, the stigma provides initial nutrients and guidance cues for pollen grain germination and tube growth. However, little is known about the genes that regulate these processes in rice (*Oryza sativa*). Here, we generate rice stigma-specific or -preferential gene expression profiles through comparing genome-wide expression patterns of hand-dissected, unpollinated stigma at anthesis with seven tissues, including seedling shoot, seedling root, mature anther, ovary at anthesis, seeds 5 d after pollination, 10-d-old embryo, 10-d-old endosperm, and suspension-cultured cells by using both 57 K Affymetrix rice whole-genome array and 10 K rice cDNA microarray. A high reproducibility of the microarray results was detected between the two different technology platforms. In total, we identified 548 genes to be expressed specifically or predominantly in the stigma papillar cells of rice. Real-time quantitative reverse transcription-polymerase chain reaction analysis of 34 selected genes all confirmed their stigma-specific expression. The expression of five selected genes was further validated by RNA in situ hybridization. Gene Ontology analysis shows that several auxin-signaling components, transcription, and stress-related genes are significantly overrepresented in the rice stigma gene set. Interestingly, most of them also share several cis-regulatory elements with known stress-responsive genes, supporting the notion of an overlap of genetic programs regulating pollination and stress/defense responses. We also found that genes involved in cell wall metabolism and cellular communication appear to be conserved in the stigma between rice and Arabidopsis (*Arabidopsis thaliana*). Our results indicate that the stigmas appear to have conserved and novel molecular functions between rice and Arabidopsis.

In flowering plants, pollination is the first major reproduction process that results in the production of seeds. The process begins with the adhesion of pollen grains to the stigmatic tissue of style. Then, the highly desiccated pollen grains are rehydrated to reach a certain water content that allows them to regain active metabolisms. Once the pollen grain cell has established its internal polarity relative to an external signal, the pollen germinates and breaches the exine wall to emit a pollen tube. The newly formed pollen tube subsequently penetrates the stigma, grows down through the transmitting tissue of the style, and ultimately reaches an ovule, allowing fertilization to take place (Lord, 2003; Sanchez et al., 2004; Swanson et al., 2004; Boavida et al., 2005).

Successful pollination requires continued communication and coordination between the pollen and stigma and is essential to maximize the seed set. The stigma, the uppermost part of the pistil, is generally considered to be a passive structure for pollen grain capture and reception, germination, and initial growth of the pollen tubes. There are two major types of stigma, the dry and the wet types, which differ by the presence or absence of the secreting exudate compounds (Edlund et al., 2004). The adhesion and germination of pollen on the wet stigma is facilitated by the presence of the exudate, but its ingredients are highly variable among species (Edlund et al., 2004; Boavida et al., 2005). Tobacco (*Nicotiana tabacum*) is a wet stigma-type plant, and its stigma has three distinct zones: an epidermis with papillae, a subepidermal secretory zone, and a zone of parenchyma ground tissue (Kandasamy and Kristen, 1987). Elimination of the secretory zone involved in producing the exudates in tobacco by expressing a chimeric stigma-specific cytotoxic gene results in female sterility, but fertility could be restored upon application of the stigma exudate of wild-type tobacco plants (Goldman et al., 1994). In species with the dry stigma, including Arabidopsis (*Arabidopsis thaliana*), kale (*Brassica oleracea*), and rice (*Oryza sativa*), the epidermis is composed of large specialized papillae cells that interact directly with the surface of pollen, and the surface of each

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papilla is covered with a waxy cuticle overlaid with a distinct proteinaceous pellicle layer (Gaude and Dumas, 1986; Zinkl et al., 1999; Ciampolini et al., 2001). In *Brassica*, the stigmatic papillae cells completely lose their ability to capture the pollen grains when using acetone to strip off both the waxy cuticle and proteinaceous stigma pellicle (Heizmann et al., 2000). In addition, protease digestion of pellicles from kale also adversely affects pollen adhesion (Stead et al., 1980; Luu et al., 1997). These studies clearly demonstrate that the stigma plays crucial roles in pollination.

Nevertheless, the molecular roles of the stigma in pollination are largely unknown. Several stigma-specific expressed proteins have been identified, a few of which are found to be directly involved in the pollen-stigma interaction. *SRK* (*S-locus receptor kinase*) encoding a glycoprotein localizes to the stigmatic plasma membrane and interacts with the pollen coat S-locus Cys-rich protein (SCR) in the self-incompatibility response in *Brassica* (Nasrallah, 2000; Takayama and Isogai, 2005). Both *SLG* (*S-locus glycoprotein*) and *SLR* (*S-locus-related protein*) are expressed in stigma papillae cells, and their products localize to the stigma cell wall in *Brassica* (Kandasamy et al., 1989; Lalonde et al., 1989; Umbach et al., 1990). Although there is some controversy as to whether SLG and SLR are required for the self-incompatibility response in *Brassica*, both of them are shown to interact with several small pollen coat proteins, and these interactions are believed to generate adhesive forces of the pollen-stigma interaction (Luu et al., 1999; Takayama and Isogai, 2005). The *pis63* gene encoding an unknown protein in *B. napus* is expressed in the stigma papillae cells, and a reduction in its transcript level results in reduced pollen germination and seed set (Kang and Nasrallah, 2001). Aquaporin-like proteins in the *Brassica* stigma may act as water channels in controlling water flow into the pollen grain from the stigma (Dixit et al., 2001). An ATP-binding cassette (ABC) transporter gene, designated as *NtWBC1* (*N. tabacum* ABC transporter of the White-Brown Complex subfamily), is highly expressed in the stigmatic secretary zone, suggesting its important role in the reproductive process (Otsu et al., 2004). Recently, a stigma-specific peroxidase from the ragwort *Senecio squalidus* was identified, and its expression was confined to the specialized epidermal cells of the stigma (McInnis et al., 2005). The activity of stigma peroxidases has long been used to determine the pistil receptivity for pollen grains, and the high levels of the peroxidase activity show that the stigma is ripe for pollination (McInnis et al., 2006). But, the function of the peroxidase in pollination still is not clear.

Additional factors have been found to be involved in pollination in several species. Auxin has been proposed to operate as a pollination signal and promote the differentiation and development of ovules in flowers of orchid of the genus *Phalaenopsis* (O'Neill, 1997). In petunia (*Petunia hybrida*), the interaction of the male gametophyte with the stigmatic tissues is accompanied by a 3-fold increase in the ethylene production and a 1.5-fold increase in the auxin (indole-3-acetic acid [IAA]) content in the pollen-pistil system within 0 to 4 h after compatible pollination, and the growth of pollen tubes in the stylar tissues is accompanied by a further increase in IAA content and a decrease in the ethylene production by stigmatic tissues (Kovaleva et al., 2002). The ethylene/auxin status of the stigma has been suggested to control the processes of adhesion, hydration, and germination of pollen grains during pollination in petunia (Kovaleva et al., 2002). In our previous study, we found that an extensive set of genes is shared between abiotic stress responses and pollination/fertilization in rice, and nearly one-half of the genes expressed preferentially in the unpollinated pistils are responsive to dehydration; the majority of them are dehydration inducible, suggesting an overlap in the genetic programs controlling between these processes (Lan et al., 2005). Temperature is a major physical factor influencing the plant reproductive phase and limits geographical distributions of plant species. High temperature has been found to cause the loss of the sporophytic self-incompatibility response in *Ipomoea fistulosa* (Prabha et al., 1982). The stigmatic receptivity of peach (*Prunus persica*) loses the capacity to sustain the pollen tube penetration into the transmitting tissue, pollen germination, and adhesion as the temperature increases during pollination (Hedhly et al., 2005).

However, the molecular mechanisms of these events involved in pollination remain obscure. It is anticipated that, given recent rapid developments in functional genomics, genome-wide identification of stigma-specific genes could provide important initial steps in dissecting molecular control of pollination. Recently, two groups have carried out the stigma-specific gene profiling of Arabidopsis using whole-genome microarray analysis (Swanson et al., 2005; Tung et al., 2005). Tung et al. (2005) have identified 115 genes predicted to be expressed specifically in the stigma epidermis after comparing transcriptional profiles of the stigmas isolated from wild-type Arabidopsis and from the transgenic plants in which cells of the stigma epidermis were specifically ablated by expression of a cellular toxin. Swanson et al. (2005) have identified 317 genes that are highly expressed in the stigma after comparing wild-type stigma, ovary, and seedling transcriptional profiles, and the cDNA library subtractive hybridization has further confirmed the results. Both the stigma gene data sets are significantly enriched in those genes involved the cell wall metabolism, cellular communication, and signal transduction-related proteins. They are thought to play roles in the adhesion, hydration, and germination of pollen grain.

Cultivated rice is by far one of the most important food crops and is also considered the model monocot plant for molecular and genetic studies. Rice is primarily an autogamous, self-pollinating plant and has the typical dry stigma. But very little is known about stigma-specific genes that are required for ensuring successful pollination in rice. In this study, we generate

rice stigma-specific or -preferential gene expression profiles by using both the commercially available 57 K Affymetrix rice whole-genome array and 10 K rice cDNA microarray (Lan et al., 2004). Gene Ontology (GO) analysis shows that several auxin-signaling components, transcription, and stress-related genes are significantly overrepresented in the rice stigma gene set. We also found that the genes involved in cell wall metabolism and cellular communication appear to be conserved in the stigmas between rice and Arabidopsis.

RESULTS

Gene Expression Profiles of Stigma versus Ovary by Two Independent Microarray Platforms

To generate stigma-specific and ovary-specific gene expression profiles, two independent microarrays were applied in this study. The stigma samples were hand collected by cutting the pistil just below the base of the plumose stigma, and the remainder of the pistil containing the style was used as the ovary samples. The important criterion for the dissection was that the stigma samples had the papillar cells but not the ovary samples.

To identify the genes preferentially expressed in the stigma, 57 K Affymetrix rice whole-genome array was used to generate the genome expression patterns across eight representative organs or tissues and suspension-cultured cells. They included seedling shoots (Sh), seedling roots (Rt), mature anthers (An), unpollinated stigmas at anthesis (St), ovaries at anthesis (Ov), seeds 5 d after pollination (5DAP), 10-d-old embryos (10EM), and 10-d-old endosperms (10EN). We performed three biological replicates for the stigma and ovary samples, respectively. The correlation coefficient value of each experiment was larger than 0.99. By using P value ≤ 0.05 and fold change ≥ 2 as cutoff to identify differentially expressed genes, 3,300 probe sets were up-regulated in the stigma compared to the ovary, and 4,125 probe sets were up-regulated in the ovary compared to the stigma (Supplemental Table S1). All microarray data are available online (http://www.ncbi.nlm.nih.gov/geo; http://plantbiol.genetics.ac.cn).

We also used the 10 K rice cDNA microarray constructed previously (Lan et al., 2004) to compare gene expression profiles of the stigma with the ovary. The gene expression profiles of the rest organs were generated previously (Lan et al., 2004, 2005). Three biological and one dye-swapped replicates were performed for the stigma versus ovary hybridizations. By using the same cutoff (fold change ≥ 2, $P < 0.05$), 453 cDNAs in total had more abundant expressions in the stigma compared to the ovary (Supplemental Table S2), and 257 cDNAs were identified as enriched in the ovary compared to the stigma (Supplemental Table S3).

The microarray results showed a high reproducibility using the two different platforms (cDNA microarray versus Affymetrix oligo array). For the 453 cDNAs preferentially expressed in the stigma identified using the 10 K rice cDNA microarray, 201 cDNAs' (using E value = 1e-20 and identity >80% as cutoff) expression patterns were confirmed by the Affymetrix GeneChip results (Supplemental Table S4), with about a 44.37% match. For the 257 cDNA clones highly expressed in the ovary, 109 cDNAs' expression patterns (using E value = 1e-20 and identity >80% as cutoff) were confirmed by the Affymetrix GeneChip results, with about a 42.41% match (Supplemental Table S5).

Identification and Functional Classification of the Stigma-Specific/-Preferential Genes

To identify rice stigma preferentially expressed genes, two statistical methods were conducted for data processing. First, Significance Analysis of Microarrays software package analysis was conducted for three biological samples replicates between the stigma and ovary. Using q value ≤ 0.05 and fold change ≥ 2 as cutoff, 3,300 probe sets were subsequently picked out, and their expression levels were significantly upregulated in the stigma compared to the ovary. We also did a normal t test and added P value for these probe sets. Second, we used the Z-score transformation normalization method to compare the gene expression levels in stigma with other tested organs or tissues and suspension-cultured cells and directly calculated significant changes in the gene expression levels between them. For Z-score transformation normalization, we used average values of the stigma and ovary separately. In total, we identified 548 genes (665 probe sets) preferentially expressed in the stigma using Z score $\geq$ 2.32 and P value ≤ 0.01(Supplemental Table S6).

Among the identified 548 genes preferentially expressed in the stigma, 410 genes had putative functions, 103 genes were assigned as expressed proteins, 23 genes were assigned as hypothetical proteins, and 12 genes had no hits when blasted against The Institute for Genomic Research rice genome annotation database (http://www.tigr.org). The 410 genes were classified into 11 groups according to their annotations and one unclassified group: transcription, cell wall related, stress/defense, signal transduction, lipid metabolism, transport, hormone related, protein metabolism, carbohydrate and energy metabolism, nucleic acid metabolism, amino acid metabolism, and unclassified. The categories are listed in the order of gene numbers in each group (Supplemental Table S7). We list 149 genes derived from 173 probe sets with putative annotated functions and their ratios of the stigma/ovary of more than 10 ($P < 0.05$) in Table I. The largest category was transcription-related proteins, including two MADS-box proteins, four zinc-finger proteins, and six Myb- or Myb-like transcription factors. These proteins accounted for nearly 15% of the 410 putative functional genes.

Table I. *Candidate genes preferentially expressed in rice stigma*

The total number of the preferentially expressed genes is 548 (665 probe sets). Expressed protein, hypothetical protein, and no hit genes are 103, 23, and 12, respectively. The table lists 149 (173 probe sets) of them with putative annotated functions, and their ratios of stigma/ovary are more than 10 ($P < 0.05$). ID, Identification.

Probe Set ID[a]	Gene ID	Description	Stigma-S[b]	Ovary-S[c]	Ratio[d]	*P* Value
Transcription						
Os.3386.1.S1_x_at	LOC_Os06g10350	Anthocyanin regulatory C1 protein	2,087.6	90.4	23.1	1.93E-04
Os.2233.1.S1_at	LOC_Os04g47080	Anthocyanin regulatory Lc protein	7,912.47	75.93	104.2	1.98E-04
Os.38982.1.S1_at	LOC_Os02g43820	AP2 domain-containing protein	1,498.93	72.97	20.5	1.67E-02
Os.49746.1.S1_at	LOC_Os01g74020	ARR1 protein like	13,525.53	908.27	14.9	3.05E-04
Os.31989.1.S1_at	LOC_Os03g02900	B3 DNA-binding domain-containing protein	3,014.57	260.47	11.6	2.72E-03
OsAffx.27272.1.S1_at	LOC_Os05g41070	bZIP transcription factor	267.53	23.2	11.5	4.17E-02
Os.46006.1.S1_at	LOC_Os07g47140	CCT motif family protein	10,524.93	313.83	33.5	8.37E-04
Os.37618.1.S1_at	LOC_Os06g04850	Homeobox-Leu zipper protein ATHB-4	1,608.2	93.23	17.2	2.78E-03
Os.2365.1.S1_at	LOC_Os06g04870	Homeobox-Leu zipper protein HAT1	9,315.13	352.67	26.4	1.31E-04
Os.49761.1.S1_at	LOC_Os03g54170	MADS-box transcription factor 34	3,492.13	276.37	12.6	6.24E-03
OsAffx.21941.1.S1_s_at	LOC_Os01g19970	myb-like transcription factor	1,679.7	165.77	10.1	1.98E-04
OsAffx.14605.1.S1_at	LOC_Os05g09020	OsWRKY67	3,840.1	229.9	16.7	1.96E-03
OsAffx.14605.1.S1_s_at	LOC_Os05g09020	OsWRKY67	3,244.13	225.6	14.4	3.32E-07
OsAffx.2905.1.S1_at	LOC_Os02g38320	Protein-binding protein	1,157.37	63.13	18.3	2.91E-04
OsAffx.11799.1.S1_at	LOC_Os01g69200	Regulatory protein	2,144.53	2.9	739.5	2.25E-03
Os.56298.1.S1_at	LOC_Os03g63810	WRKY transcription factor 14	2,149.4	21.93	98	5.89E-04
OsAffx.17417.1.S1_x_at	LOC_Os09g31140	Zinc finger protein F15B8.140, Arabidopsis	374.63	16.3	23	8.56E-03
Os.49270.1.S1_at	LOC_Os06g40960	Zinc finger protein	5,652.4	172.57	32.8	1.37E-03
Cell wall related						
Os.53291.1.S1_at	LOC_Os03g64280	1-Aminocyclopropane-1-carboxylate oxidase	861.17	85.33	10.1	7.57E-04
Os.27155.1.S1_at	LOC_Os06g15170	3-Ketoacyl-CoA synthase	2,845.73	76.87	37	1.19E-02
Os.2367.1.S1_at	LOC_Os03g21820	α-Expansin 10 precursor	11,840.7	658.43	18	5.25E-04
Os.31669.1.S1_at	LOC_Os01g53370	Anthocyanidin 5,3-*O*-glucosyltransferase	475.63	5.57	85.4	6.27E-03
Os.31669.1.S1_x_at	LOC_Os01g53370	Anthocyanidin 5,3-*O*-glucosyltransferase	667.57	10.43	64	9.77E-03
Os.8842.1.S1_at	LOC_Os07g32620	Anthocyanidin 5,3-*O*-glucosyltransferase	15,546.97	288.1	54	2.47E-04
Os.57078.1.S1_at	LOC_Os05g45200	Anthocyanidin 5,3-*O*-glucosyltransferase	1,105.83	47.2	23.4	1.24E-02
OsAffx.23995.2.S1_x_at	LOC_Os01g71094	Basic 7S globulin 2 precursor	349.03	4.27	81.8	3.36E-02
Os.27968.1.S1_x_at	LOC_Os01g71094	Basic 7S globulin 2 precursor	2,661.17	184.47	14.4	1.16E-03
Os.27968.1.S1_at	LOC_Os01g71094	Basic 7S globulin 2 precursor	3,755.6	271.67	13.8	5.83E-03
Os.2075.1.S1_at	LOC_Os01g19220	β-D-Xylosidase	6,110.4	567.4	10.8	1.70E-04
Os.14358.4.S1_x_at	LOC_Os02g12730	β-Galactosidase precursor	6,569.7	243.7	27	7.94E-04
Os.14358.1.S1_at	LOC_Os02g12730	β-Galactosidase precursor	1,490.43	71.67	20.8	3.07E-04
Os.2322.1.S1_at	LOC_Os10g33250	CER1	28,284.37	469.33	60.3	4.07E-03
Os.2322.2.S1_at	LOC_Os10g33250	CER1	1,049.97	32.73	32.1	2.42E-02
OsAffx.15853.1.S1_x_at	LOC_Os06g39970	CESA11, cellulose synthase	252.67	15	16.8	1.77E-02
OsAffx.15853.1.S1_at	LOC_Os06g39970	CESA11, cellulose synthase	200.13	15.33	13.1	5.18E-03
OsAffx.22999.1.S1_at	LOC_Os07g32710	D-Genomic expressed protein	19,698.87	292.03	67.5	4.54E-03
OsAffx.4968.1.S1_at	LOC_Os06g30090	DNA-binding protein	325.67	2.43	133.8	4.58E-03
Os.5804.1.S1_at	LOC_Os11g40210	DNA-binding protein	1,662.97	129.93	12.8	6.56E-04
OsAffx.2049.2.S1_x_at	LOC_Os01g21310	dTDP-Glc 4-6-dehydratase-like protein	534.87	33.43	16	4.85E-02
Os.26486.1.S1_at	LOC_Os09g25850	gl1 protein	19,971.97	353.67	56.5	2.77E-03
OsAffx.30743.1.S1_at	LOC_Os01g14900	Glycerol-3-P acyltransferase 1	135.2	5.87	23	5.01E-03
Os.32123.1.S1_at	LOC_Os01g02930	Glycosyltransferase	7,300.53	480.7	15.2	1.54E-03
Os.53164.1.S1_at	LOC_Os06g18010	Hydroquinone glucosyltransferase	7,375.97	115	64.1	6.97E-04
Os.15570.1.S1_at	LOC_Os07g32630	Hydroquinone glucosyltransferase	10,598	395.8	26.8	1.99E-04
OsAffx.31322.1.S1_at	LOC_Os11g36240	Pectinesterase precursor	6,228.2	1.53	4,061.9	9.86E-04
Os.6192.1.S1_at	LOC_Os03g02460	Retinol dehydrogenase 12	10,156.47	721.63	14.1	1.60E-06
Stress/defense						
Os.4377.1.S1_at	LOC_Os02g46970	4-Coumarate-CoA ligase 2	4,725.17	28.87	163.7	1.03E-03
Os.46537.1.S1_at	LOC_Os10g04429	Alcohol acyl transferase	336.03	6.07	55.4	2.42E-03
Os.23215.1.S1_at	LOC_Os06g24404	Anther-specific Pro-rich protein APG precursor	4,965.7	247.37	20.1	2.43E-03
Os.22594.2.S1_at	LOC_Os01g03390	Bowman-Birk-type bran trypsin inhibitor precursor	206.1	9.63	21.4	1.29E-02

(*Table continues on following page.*)

Table I. (*Continued from previous page.*)

Probe Set ID[a]	Gene ID	Description	Stigma-S[b]	Ovary-S[c]	Ratio[d]	*P* Value
Os.22594.1.S1_at	LOC_Os01g03390	Bowman-Birk-type bran trypsin inhibitor precursor	20,176.87	1,726.13	11.7	4.64E-03
Os.3121.1.S1_at	LOC_Os10g28050	Chitinase 2	11,909.67	287.67	41.4	7.30E-03
Os.47136.1.A1_x_at	LOC_Os01g27360	D-Genomic glutathione *S*-transferase GSTF1	1,140.33	36.13	31.6	2.45E-02
Os.14539.1.S1_at	LOC_Os07g01600	Disease resistance response protein 206	10,803.27	662.5	16.3	2.94E-03
Os.12922.1.S1_at	LOC_Os09g36700	Extracellular ribonuclease LE precursor	9,753.43	146.17	66.7	1.47E-04
Os.46551.1.S1_at	LOC_Os10g17260	Flavonoid 3-monooxygenase	3,769.07	16.27	231.7	6.04E-03
Os.25651.1.S1_at	LOC_Os01g27360	Glutathione *S*-transferase GSTF1	2,437.67	59.23	41.2	7.15E-03
OsAffx.13105.1.S1_at	LOC_Os03g29250	ids4-like protein	134.93	9.43	14.3	6.16E-03
Os.51747.1.S1_at	LOC_Os03g29250	ids4-like protein	1,188.83	94.13	12.6	2.91E-03
OsAffx.14039.1.S1_s_at	LOC_Os04g30040	Jacalin-like lectin domain containing protein	348.73	5.33	65.4	7.62E-03
OsAffx.25096.1.S1_at	LOC_Os03g15360	Leucoanthocyanidin reductase	4,015.07	294.57	13.6	9.87E-04
Os.42490.1.S1_at	LOC_Os01g62070	Metal tolerance protein C3	28,270.2	199.37	141.8	4.92E-03
Os.42490.1.S1_x_at	LOC_Os01g62070	Metal tolerance protein C3	27,456.03	206.4	133	3.13E-03
OsAffx.10363.1.S1_at	LOC_Os06g03500	NBS-LRR disease resistance protein	225.27	17.27	13	2.59E-02
Os.12851.1.S1_at	LOC_Os01g14670	Nectarin-1 precursor	6,357.33	141.37	45	6.85E-04
Os.12761.1.S1_at	LOC_Os03g46060	Osmotin-like protein OSML13 precursor	19,564.23	702.03	27.9	2.60E-03
Os.52536.1.S1_at	LOC_Os04g43800	Phenyl-Ala ammonia-lyase	6,313.3	20.2	312.5	2.92E-03
Os.50455.1.S1_at	LOC_Os06g40170	Phospholipase D-α-2	3,267.17	166.77	19.6	9.06E-05
Os.11575.1.S1_a_at	LOC_Os03g46440	Regulatory protein NPR1	1,400.97	120.6	11.6	9.44E-03
OsAffx.13793.1.S1_at	LOC_Os04g12840	Resistance protein	1,537.77	142.13	10.8	4.86E-05
Os.10600.1.S1_a_at	LOC_Os01g25820	Respiratory burst oxidase 2	12,428.1	1,231.33	10.1	2.68E-03
Os.57186.1.S1_at	LOC_Os05g27590	Wound-induced protein WI12-containing protein	1,239.17	45.27	27.4	3.73E-02
Signal transduction						
OsAffx.13408.1.S1_at	LOC_Os03g48560	BGGP β-1-3-galactosyl-*O*-glycosyl-glycoprotein	350.6	24.93	14.1	7.61E-03
OsAffx.13408.1.S1_x_at	LOC_Os03g48560	BGGP β-1-3-galactosyl-*O*-glycosyl-glycoprotein	224.77	19.97	11.3	1.68E-03
OsAffx.24314.1.S1_at	LOC_Os02g18930	Calcineurin B-like protein 4	2,628.87	24.77	106.1	4.50E-03
Os.15639.1.S1_at	LOC_Os06g40370	CBL-interacting Ser/Thr-protein kinase 24	7,217.23	666.6	10.8	3.66E-03
Os.48846.1.S1_at	LOC_Os09g24840	GAST1 protein precursor	3,067.53	136.57	22.5	1.28E-04
Os.17201.1.S1_at	LOC_Os07g38810	Lectin receptor-type protein kinase	3,123.2	82.57	37.8	3.28E-03
Os.6360.1.S1_at	LOC_Os08g02996	Lectin-like receptor kinase 1	4,453.83	85.53	52.1	1.53E-03
Os.6360.2.S1_x_at	LOC_Os08g02996	Lectin-like receptor kinase 1	4,304.07	162.17	26.5	2.10E-03
OsAffx.30716.1.S1_x_at	LOC_Os10g38960	Lectin-like receptor kinase 7	3,306.8	244.23	13.5	3.84E-03
Os.12660.1.S2_at	LOC_Os07g38800	Lectin-like receptor kinase 7	2,745.87	27.73	99	2.88E-03
Os.12660.1.S1_at	LOC_Os07g38800	Lectin-like receptor kinase 7	938.63	16.2	57.9	1.14E-02
Os.26943.1.S1_at	LOC_Os09g32840	Nucleotide pyrophosphatase/phosphodiesterase	3,333.23	205.1	16.3	1.96E-03
OsAffx.29386.1.S1_x_at	LOC_Os08g27810	OsWAK115	1,312.67	121.3	10.8	1.52E-02
OsAffx.6309.1.S1_at	LOC_Os09g16980	OsWAK86	2,528.73	9.33	270.9	2.87E-03
Os.54625.1.S1_at	LOC_Os12g42020	Protein kinase PVPK-1	7,055.77	441.67	16	2.73E-04
Os.44814.1.S1_at	LOC_Os06g03610	Protein kinase	85.97	4.37	19.7	1.29E-02
OsAffx.17892.1.S1_at	LOC_Os09g25540	Receptor-like protein kinase precursor	124.07	6.3	19.7	3.54E-02
OsAffx.14892.1.S1_at	LOC_Os05g30740	SRC2	823.27	54.3	15.2	6.54E-04
Lipid metabolism						
Os.5295.1.S1_at	LOC_Os08g10010	Acyl-desaturase, chloroplast precursor	12,530.77	158.63	79	4.74E-03
Os.30537.1.S1_at	LOC_Os03g01820	C-4 methylsterol oxidase	7,759.1	306.77	25.3	1.48E-04
Os.57035.1.S1_at	LOC_Os01g21300	Catalytic/hydrolase	605.6	45.2	13.4	1.21E-02
Os.54454.1.S1_at	LOC_Os11g32650	Chalcone synthase	765.67	4.03	189.8	1.61E-02
Os.57449.1.S1_x_at	LOC_Os11g32650	Chalcone synthase	10,288	150.9	68.2	3.56E-03
Os.37295.1.S1_at	LOC_Os11g02440	Chalcone-flavonone isomerase	2,854.53	13.1	217.9	7.51E-03
Os.6354.1.S1_s_at	LOC_Os12g02370	Chalcone-flavonone isomerase	13,196.17	228.1	57.9	1.56E-04
Os.6354.1.S1_at	LOC_Os12g02370	Chalcone-flavonone isomerase	5,023.07	118.8	42.3	4.68E-04
Os.53034.1.A1_at	LOC_Os11g18194	Cycloartenol synthase	10,955.6	23.7	462.3	4.83E-03
Os.52235.1.S1_at	LOC_Os02g21810	Cyt P450	2,250.9	4.37	515.5	6.50E-03
Os.25621.2.S1_at	LOC_Os12g16720	Cyt P450	16,932.33	546.27	31	2.06E-03
Os.53717.1.S1_at	LOC_Os06g01250	Cyt P450	13,610.93	88.73	153.4	2.18E-04

(*Table continues on following page.*)

Table I. (*Continued from previous page.*)

Probe Set ID[a]	Gene ID	Description	Stigma-S[b]	Ovary-S[c]	Ratio[d]	*P* Value
Os.39637.1.A1_s_at	LOC_Os09g17000	Glycerophosphoryl diester phosphodiesterase family protein	6,529.73	75.93	86	3.88E-03
Os.39637.1.A1_at	LOC_Os09g17000	Glycerophosphoryl diester phosphodiesterase family protein	4,584.1	73.8	62.1	2.93E-03
Os.47906.1.A1_at	LOC_Os06g49770	Lipid-binding protein	7,864.5	210.5	37.4	3.39E-03
OsAffx.32067.1.S1_x_at	LOC_Os12g37320	Lipoxygenase 2.2, chloroplast precursor	194.63	7.5	26	4.50E-03
Os.41251.1.A1_at	LOC_Os01g43140	Triacylglycerol lipase	2,613.6	244.57	10.7	3.00E-04
OsAffx.14502.1.S1_at	LOC_Os05g02350	Type I inositol-1,4,5-trisphosphate 5-phosphatase CVP2	393.47	12.6	31.2	1.50E-03
Os.29290.1.S1_at	LOC_Os01g08780	Type I inositol-1,4,5-trisphosphate 5-phosphatase CVP2	4,496.2	65.27	68.9	3.62E-03
Transport						
Os.17440.1.S1_at	LOC_Os04g44610	ABC transporter C05D10.3 in chromosome III	9,093.1	674.9	13.5	5.79E-04
Os.55221.1.S1_at	LOC_Os08g43120	ABC transporter	11,133.57	462.03	24.1	1.16E-02
OsAffx.3427.1.S1_at	LOC_Os03g37960	Acyl-CoA-binding protein	782.93	58.17	13.5	4.37E-03
Os.26802.2.S1_at	LOC_Os12g08090	Amino acid transporter	18,660.77	1,722.8	10.8	4.13E-04
OsAffx.19991.1.S1_at	LOC_Os12g36660	Antiporter/drug transporter/transporter	1,091.07	41.33	26.4	5.61E-03
OsAffx.24678.3.S1_at	LOC_Os02g41860	Aquaporin PIP2.2	1,547.47	138.83	11.1	2.14E-03
OsAffx.24678.2.S1_at	LOC_Os02g41860	Aquaporin PIP2.2	790.03	73.83	10.7	8.10E-03
Os.49307.1.S1_at	LOC_Os12g22284	ABC subfamily G member 2	3,085.93	7.1	434.6	6.65E-04
Os.52570.1.S1_at	LOC_Os12g44110	ligA	12,413.13	851.5	14.6	1.50E-02
OsAffx.3105.1.S1_at	LOC_Os02g58530	Major facilitator superfamily protein	237.23	11.8	20.1	2.45E-02
Os.27833.1.S1_at	LOC_Os12g32940	Major myo-inositol transporter iolT	2,600.73	98.1	26.5	5.41E-03
OsAffx.20797.1.S1_at	LOC_Os10g02340	Peptide transporter PTR2	275	12.63	21.8	4.68E-03
Os.46447.1.S1_at	LOC_Os10g33210	Peptide transporter PTR2	1,797.1	113.97	15.8	5.30E-03
Os.11547.1.S1_s_at	LOC_Os06g48030	Peroxidase 16 precursor	9,654.5	622.3	15.5	5.94E-05
Os.5843.1.S1_at	LOC_Os06g46799	Peroxidase 39 precursor	11,243.27	277.83	40.5	3.39E-03
Os.53303.1.S1_at	LOC_Os06g46799	Peroxidase 39 precursor	1,034.43	56.8	18.2	5.73E-03
Os.10754.1.S1_at	LOC_Os03g06520	Sulfate transporter 3.1	7,211.23	668.47	10.8	3.14E-03
Hormone related						
Os.27822.2.S1_at	LOC_Os04g47520	Auxin-independent growth promoter	1,048.63	101.87	10.3	2.04E-02
OsAffx.21458.1.S1_at	LOC_Os01g46030	Brassinosteroid insensitive 1-associated receptor kinase 1 precursor	278.63	17.87	15.6	6.22E-03
OsAffx.28262.1.S1_at	LOC_Os07g04520	Brassinosteroid insensitive 1-associated receptor kinase 1 precursor	939.93	8.37	112.3	1.19E-02
OsAffx.15447.1.S1_at	LOC_Os06g16000	Cytokinin-*O*-glucosyltransferase 3	2,037.37	186.13	10.9	6.74E-04
Os.36449.1.S1_at	LOC_Os01g57610	IAA-amido synthetase GH3.1	10,154.47	533.07	19	4.14E-04
OsAffx.32069.1.S1_at	LOC_Os12g37490	*N*-Acetyltransferase	405.6	22.13	18.3	8.10E-04
Os.55720.1.S1_at	LOC_Os04g56690	OsSAUR23	997.73	47.67	20.9	3.74E-03
OsAffx.30169.1.S1_at	LOC_Os09g37430	OsSAUR48	781.37	51.53	15.2	1.61E-03
Os.51408.1.S1_at	LOC_Os09g37490	OsSAUR54	1,076.63	64.17	16.8	1.47E-02
Os.50785.1.S1_at	LOC_Os09g37500	OsSAUR55	5,390.27	317.77	17	4.96E-03
Protein metabolism						
Os.54875.1.S1_at	LOC_Os05g04584	3-*N*-debenzoyl-2-deoxytaxol *N*-benzoyltransferase	337.53	19.3	17.5	8.62E-04
OsAffx.24010.1.S1_x_at	LOC_Os01g72020	BOP2	806.33	30.57	26.4	2.00E-03
Os.49276.1.S1_at	LOC_Os01g72020	BOP2	1,908.9	162.2	11.8	1.03E-03
Os.52793.1.S1_at	LOC_Os04g43840	Carboxy-lyase	5,289.97	30.97	170.8	2.02E-03
Os.10714.1.S1_s_at	LOC_Os04g38450	γ-Glutamyltranspeptidase 1 precursor	1,0921.73	330.83	33	6.63E-04
Os.10728.1.S1_at	LOC_Os02g46260	Ser carboxypeptidase 1 precursor	23,058.93	857.67	26.9	3.13E-03
OsAffx.10889.1.S1_x_at	LOC_Os01g03170	Seven in absentia protein family protein	4,477.57	409.87	10.9	2.57E-04
Os.3768.1.S1_at	LOC_Os02g44590	Subtilisin-like protease precursor	11,949.97	366.4	32.6	5.07E-03
Os.8271.2.S1_x_at	LOC_Os01g67500	Ubiquitin-protein ligase	1,791.4	174.73	10.3	8.17E-03
OsAffx.13747.1.S1_at	LOC_Os04g10360	Xylem Ser proteinase 1 precursor	4,292.53	12.23	350.9	6.60E-04
Carbohydrate and energy metabolism						
Os.53178.2.S1_at	LOC_Os05g47840	ATIPT1	124.5	10.23	12.2	9.57E-03
OsAffx.28287.1.S1_at	LOC_Os07g05380	ATPase	1,148.87	33.53	34.3	6.85E-03
OsAffx.5436.1.S1_at	LOC_Os07g25540	Disulfide oxidoreductase/monooxygenase/ oxidoreductase	197.67	8.37	23.6	1.74E-03

(*Table continues on following page.*)

Table I. (*Continued from previous page.*)

Probe Set ID[a]	Gene ID	Description	Stigma-S[b]	Ovary-S[c]	Ratio[d]	*P* Value
OsAffx.31989.1.S1_at	LOC_Os12g32750	Disulfide oxidoreductase/monooxygenase/ oxidoreductase	2,854.47	149.03	19.2	6.31E-04
Os.18223.1.S1_s_at	LOC_Os12g32750	Disulfide oxidoreductase/monooxygenase/ oxidoreductase	7,562.97	747.77	10.1	3.81E-04
OsAffx.13016.1.S1_at	LOC_Os03g25150	Flavonoid 3,5-hydroxylase 2	1,361.83	57.23	23.8	4.06E-02
OsAffx.13016.1.S1_x_at	LOC_Os03g25150	Flavonoid 3,5-hydroxylase 2	1,470.4	78.63	18.7	4.62E-02
OsAffx.27142.1.S1_at	LOC_Os05g33644	Hydrolase	1,714.27	93.2	18.4	2.38E-02
Os.46268.1.S1_at	LOC_Os10g02390	NAD(P)H-dependent oxidoreductase	7,951.73	3.63	2,188.6	6.30E-03
Nucleic acid metabolism						
Os.855.1.S1_at	LOC_Os01g03730	Nuclease PA3	5,603.9	85.67	65.4	1.12E-02
Os.55424.2.S1_at	LOC_Os02g50740	Nucleotide binding protein	2,223.23	164.1	13.5	8.77E-05
Amino acid metabolism						
Os.27876.1.S1_x_at	LOC_Os08g04540	Aromatic-L-amino acid decarboxylase	3,587.43	19.3	185.9	1.49E-02
OsAffx.5702.1.S1_s_at	LOC_Os08g04560	Aromatic-L-amino acid decarboxylase	23,981.57	192.23	124.8	9.93E-04
Unclassified						
Os.48933.1.S1_at	LOC_Os05g37880	axi 1-like protein	12,167.7	1,092.03	11.1	1.32E-03
OsAffx.3289.1.S1_at	LOC_Os03g21450	Bromodomain-containing protein	433.77	23.43	18.5	2.21E-02
Os.28450.1.S1_at	LOC_Os01g70730	FLP1	3,929.67	103.73	37.9	1.54E-04
Os.6003.1.S1_x_at	LOC_Os09g26310	Hypro1	3,628.77	109.37	33.2	4.80E-05
Os.51638.1.S1_s_at	LOC_Os09g26310	Hypro1	4,551.53	161	28.3	4.31E-04
Os.49681.1.S1_at	LOC_Os03g07140	Male sterility protein 2	2,714.93	42.37	64.1	4.57E-02
Os.24786.1.S1_s_at	LOC_Os04g48870	Nitrilase-associated protein	4,117.03	126.8	32.5	2.12E-02
OsAffx.26533.1.S1_at	LOC_Os04g48870	Nitrilase-associated protein	4,745.7	152.2	31.2	1.16E-02
OsAffx.12915.1.S1_at	LOC_Os03g18300	Pherophorin-like protein	570.47	21.47	26.6	1.84E-03
Os.46488.1.S1_at	LOC_Os10g05790	Pro-rich protein	28,988.2	512.93	56.5	1.96E-03
OsAffx.16391.1.S1_at	LOC_Os07g24100	Retrotransposon protein	707.07	5.73	123.3	1.28E-04
Os.28346.5.S1_x_at	LOC_Os03g63124	Retrotransposon protein, unclassified	242	23.33	10.4	1.57E-02
Os.50793.1.S1_at	LOC_Os12g01900	Retrotransposon protein, unclassified	621.7	6.53	95.2	7.10E-03
Os.55132.1.S1_at	LOC_Os03g26990	VQ motif family protein	222.07	20.03	11.1	1.21E-02

[a]The gene probes of 57 K Affymetrix rice GeneChip. ID, Identity. [b]The gene's average signal intensity in stigma derived from three biological replicates of 57 K Affymetrix rice GeneChip. Stigma-S, Stigma signal. [c]The gene's average signal intensity in ovary derived from three biological replicates of 57 K Affymetrix rice GeneChip. Ovary-S, Ovary signal. [d]The ratios of stigma/ovary.

In the second category, including putative cell wall-localized enzymes (e.g. α-expansin, pectinesterase, and peroxidase), cellulose synthase, glycerol-3-P acyltransferase, and UDP-glucosyltransferase, the third category consisted of proteins that were potentially involved in stress/defense responses, such as glutathione *S*-transferase, phospholipase D, and wound-induced protein WI12. Many signal transduction- and transport-related proteins were enriched in the stigma. There was a large percentage of genes that may be related to lipid metabolism, including lipid binding protein, triacylglycerol lipase, and fatty acid elongase. The auxin signaling related proteins were identified to be highly expressed in the stigma, including auxin-responsive factor, five members of the auxin-responsive small auxin-up RNA (SAUR) gene family, and the highly expressed IAA-amido synthetase *GH3.1* gene (see Table I; Supplemental Table S7).

GO analysis of the rice stigma-preferential genes was performed, and the biological process term enrichment status and hierarchy are shown in Supplemental Figure S1. The results showed that the DNA-dependant transcription may play an important role in the stigma, and the biological processes that respond to hormone stimuli, especially to auxin, were overrepresented and seemed to be more significant in the rice stigma-specific or -preferential gene data set.

Comparison of the Stigma Preferentially Expressed Genes in Rice and Arabidopsis

We compared the stigma preferentially expressed genes in rice with that identified in Arabidopsis (Tung et al., 2005). We extracted the protein sequences for 115 Arabidopsis genes, blasted them against the rice Affymetrix consensus sequence database (https://www.affymetrix.com/analysis/netaffx/index.affx), and filtered 3,504 probe sets using E value $\leq$ 1e-4. We found 45 Arabidopsis genes hitting 92 rice probe sets with the stigma preferentially expression pattern (Supplemental Table S8). Meanwhile, we also blasted the sequences of the 665 rice probe sets against the Arabidopsis whole-genome protein sequence database (http://www.arabidopsis.org/Blast/index.jsp) and found that 90 rice probe sets (83 genes) were similar to 42 Arabidopsis stigma-specific/preferentially expressed genes (Supplemental Table S8). From both BLAST results, we found that they matched to each other well. The gene annotation showed that the majority of them encoded the cell wall-related and signal transduction-related

proteins. We also listed the number of the stigma-preferential genes unique to rice (465) and Arabidopsis (70).

Validation of the Microarray Results

Real-time quantitative reverse transcription (qRT)-PCR analysis was employed to validate the candidate genes. In total, 34 of the identified genes preferentially expressed in stigma were selected, including 30 putative function genes belonging to different classification groups, two hypothetical proteins, one expressed protein, and one unknown function gene. The signal intensity range of the selected genes was from 107.8 to 2,8284.3 and the ratios (stigma versus ovary) from 2.6 to 2,188.5 (see Supplemental Table S1). To confirm whether these genes were stigma-specific by qRT-PCR analysis, the expression of the 34 candidate genes was compared between the stigma and the ovary, anther, seeding-shoot, or flag leaf (heading stage) samples (Table II). The results showed that their expression patterns detected by 57 K Affymetrix rice whole-genome array were in good correlation with those obtained by qRT-PCR.

Confirmation of the Candidate Stigma-Specific Genes by RNA in Situ Hybridization

To further examine the expression of the candidate stigma-specific genes, we selected five genes to perform RNA in situ hybridization, including a Ser carboxypeptidase 1 precursor gene (LOC_Os02g46260), an

Table II. *Verification of microarray results by real-time quantitative RT-PCR*

ID, Identification.

Probe Set ID[a]	Gene ID	Description	Stigma/Ovary[b]	Stigma/Anther[c]	Stigma/Shoot[d]	Stigma/Leaf[e]
Os.12922.1.S1_at	LOC_Os08g10010	Acyl-desaturase, chloroplast precursor	182.34 ± 26.76	165.43 ± 23.822	2.29 ± 0.053	4,239.26 ± 1,003.77
Os.2696.1.S1_at	LOC_Os04g15840	α-Expansin 1 precursor	1.46 ± 0.033	3.43 ± 0.25	10.18 ± 1.399	2.07 ± 0.089
Os.31669.1.S1_at	LOC_Os01g53370	Anthocyanidin 5,3-*O*-glucosyltransferase	86.66 ± 1.7	21.05 ± 0.282	23.54 ± 0.327	482.61 ± 13.111
OsAffx.5702.1.S1_s_at	LOC_Os08g04560	Aromatic-L-amino acid decarboxylase	2,087.29 ± 265.908	369.34 ± 36.352	68.17 ± 4.788	69.3 ± 4.887
OsAffx.20044.1.S1_at	LOC_Os12g40080	B3 DNA-binding domain containing protein	12,823.5 ± 1,586.625	1,647.15 ± 159.426	1,204.98 ± 111.692	689.49 ± 58.868
OsAffx.23995.2.S1_x_at	LOC_Os01g71094	Basic 7S globulin 2 precursor	32.59 ± 0.873	7.53 ± 0.117	25.4 ± 0.632	6.65 ± 0.097
Os.22594.2.S1_at	LOC_Os01g03390	Bowman-Birk-type bran trypsin inhibitor precursor	24.59 ± 1.408	11.7 ± 0.514	14.42 ± 0.688	67.8 ± 5.111
Os.2322.1.S1_at	LOC_Os10g33250	CER1	319.88 ± 40.499	38.1 ± 3.04	1,362.35 ± 216.136	1,308.77 ± 206.473
Os.27065.1.S1_a_at	LOC_Os01g59180	Cyclin-like F box	4.77 ± 0.059	14 ± 0.295	6.53 ± 0.098	25.4 ± 0.657
Os.51106.1.S1_at	LOC_Os06g14540	Endoglucanase 1 precursor	252.24 ± 25.059	188.66 ± 17.754	130.53 ± 11.419	34.2 ± 2.167
OsAffx.28068.1.S1_at	LOC_Os06g42730	Esterase/lipase/thioesterase	4.03 ± 0.053	2.26 ± 0.017	2.3 ± 0.018	6.33 ± 0.111
OsAffx.21178.1.S1_x_at	LOC_Os01g68140	Expressed protein	97.87 ± 9.342	1,921.16 ± 303.246	4,341.75 ± 759.941	21,884.94 ± 4,580.042
Os.5295.1.S1_at	LOC_Os09g36700	Extracellular ribonuclease LE precursor	1.34 ± 0.002	10.64 ± 0.161	1.53 ± 0.004	16.49 ± 0.296
Os.29814.1.S1_at	LOC_Os03g62860	Glucan endo-1,3-β-glucosidase A6 precursor	119.47 ± 6.068	53.08 ± 2.238	134.61 ± 7.007	52.28 ± 2.196
Os.50860.1.S1_at	LOC_Os09g27680	Hypothetical protein	19.23 ± 0.535	2.44 ± 0.02	9.97 ± 0.215	1.68 ± 0.008
Os.55613.1.S1_at	LOC_Os01g65692	Hypothetical protein	96.45 ± 3.506	55.1 ± 1.758	55.83 ± 1.787	50.6 ± 1.58
Os.12660.1.S2_at	LOC_Os07g38800	Lectin-like receptor kinase 7	9.95 ± 0.143	6.95 ± 0.085	10.67 ± 0.158	3.35 ± 0.025
Os.47906.1.A1_at	LOC_Os06g49770	Lipid-binding protein	117.27 ± 8.537	46.61 ± 2.735	16.09 ± 0.683	2.74 ± 0.042
Os.31652.1.S1_at	LOC_Os01g06320	myb-like DNA-binding domain protein	37.96 ± 0.79	6.3 ± 0.066	148.69 ± 4.255	79.49 ± 1.99
Os.46268.1.S1_at	LOC_Os10g02390	NAD(P)H-dependent oxidoreductase	51.29 ± 0.696	4.37 ± 0.022	5.15 ± 0.029	4.95 ± 0.027
Os.10675.1.A1_at	LOC_Os03g46060	Osmotin-like protein OSML13 precursor	11.46 ± 0.017	26.72 ± 0.054	34.75 ± 0.076	52.39 ± 0.129
OsAffx.30169.1.S1_at	LOC_Os09g37430	OsSAUR48	15.39 ± 1.334	43.55 ± 5.216	19.91 ± 1.889	53.37 ± 6.738
Os.51408.1.S1_at	LOC_Os09g37490	OsSAUR54	105.91 ± 2.819	2.21 ± 0.01	2.79 ± 0.016	15.13 ± 0.235
OsAffx.3726.1.S1_at	LOC_Os04g05050	Pectate lyase 8 precursor	14.62 ± 0.457	5.22 ± 0.101	6.46 ± 0.141	608.27 ± 45.45
Os.53102.1.S1_s_at	LOC_Os09g21000	Potassium transporter 12	38.62 ± 0.749	8.77 ± 0.101	12.95 ± 0.176	12.75 ± 0.172
Os.10728.1.S1_at	LOC_Os02g46260	Ser carboxypeptidase 1 precursor	42.35 ± 0.681	43.97 ± 0.714	8.72 ± 0.081	49.59 ± 0.831
Os.12761.1.S1_at	LOC_Os02g46260	Ser carboxypeptidase 1 precursor	125.09 ± 21.094	500.45 ± 108.954	71.65 ± 10.678	184.39 ± 33.617
Os.41415.1.S1_at	LOC_Os01g40499	Ser/Thr-protein kinase receptor precursor	14.03 ± 1.286	5.34 ± 0.31	11.06 ± 0.921	4.3 ± 0.217
OsAffx.14892.1.S1_at	LOC_Os05g30740	SRC2	931.33 ± 5.402	3,901.73 ± 27.372	111.49 ± 0.446	126.66 ± 0.52
Os.189.1.S1_at	LOC_Os08g43160	TCP family transcription factor containing protein	1.99 ± 0.006	1.87 ± 0.005	5.43 ± 0.042	2.79 ± 0.013
Os.52592.1.S1_at	LOC_Os02g49480	Transcription factor BIM1	8.67 ± 0.452	7.61 ± 0.373	6.02 ± 0.261	9.38 ± 0.507
Os.8271.2.S1_x_at	LOC_Os01g67500	Ubiquitin-protein ligase	11.65 ± 0.081	25.74 ± 0.236	26.56 ± 0.246	45.2 ± 0.486
Os.56298.1.S1_at	LOC_Os03g63810	WRKY transcription factor 14	296.26 ± 15.558	95.22 ± 4.002	5.54 ± 0.087	5.51 ± 0.087
Os.23805.1.S1_at	AK071040	Unknown	63.34 ± 1.016	77.33 ± 1.3	301.67 ± 6.661	1,212.9 ± 33.31

[a]The gene probes of 57 K Affymetrix rice GeneChip. [b]Real-time RT-PCR results ± the SDS for stigma/ovary. [c]Real-time RT-PCR results ± the SDS for stigma/anther. [d]Real-time RT-PCR results ± the SDS for stigma/shoot (seedling shoot). [e]Real-time RT-PCR results ± the SDS for stigma/leaf (mature leaf).

extracellular ribonuclease LE precursor gene (LOC_Os09g36700), a putative CER1 gene (LOC_Os10g33250), a metal tolerance gene C3 (LOC_Os01g62070), and an unknown function gene (AK071040). They exhibited relatively high hybridization signals in the stigma microarray data sets, ensuring that their transcripts could be detected by the RNA in situ hybridization technology. A CCCH-type zinc finger protein gene (LOC_Os01g09620) expressed in the entire mature pistil according to the microarray results was used as a reference. Longitudinal sections through the center of pistil just before pollination were used for all the hybridizations. The results showed that the five selected genes exhibited unique expression patterns in the stigma papilla cells (Fig. 1) and that the hybridization signals of the examined genes were in good correlation with their intensities in 57 K Affymetrix rice GeneChip. Taken together, our results showed that the 548 genes preferentially expressed in the stigma thus identified represented good candidates for the stigma-specific genes in rice.

cis-Acting Regulatory Element Analyses of the Candidate Stigma-Specific Genes

To identify possible cis-acting regulatory elements responsible for the transcription regulation of the candidate stigma-specific genes, we first did the hierarchical clustering analysis and found that nearly 35% of the identified genes (193) were mainly located in three clades (Supplemental Fig. S2). The common features were that all the genes in the three clades were specific or highly expressed in the stigma, and the correlation coefficients of the three clusters were 0.9277, 0.9389, and 0.8263, respectively. We named the three clades as Cluster I, II, and III, respectively. Then, 1,000-bp regions located in the upstream of the start codons of the genes from the three clusters were used for analysis. Several conserved motifs were subsequently identified using the MEME (Multiple Em for Motif Elicitation)/MAST (Motif Alignment and Search Tool) system (Bailey and Elkan, 1994; Bailey and Gribskov, 1998). Figure 2 shows the top three motifs identified for each cluster. The E values of the three motifs are: cluster I, 3.5e + 004, 3.3e + 006, and 7.3e + 001, respectively; cluster II, 1.1e + 006, 2.8e + 003, and 2.2e + 005, respectively; and cluster III, 7.5e + 005, 3.3e + 006, and 1.2e + 007, respectively. The common features of the cis-acting regulatory elements we identified from the conserved motifs using the PLACE database were mostly related to stress or defense responses (Prestridge, 1991; Higo et al., 1999). A large percentage of the genes in cluster I had the same motif named

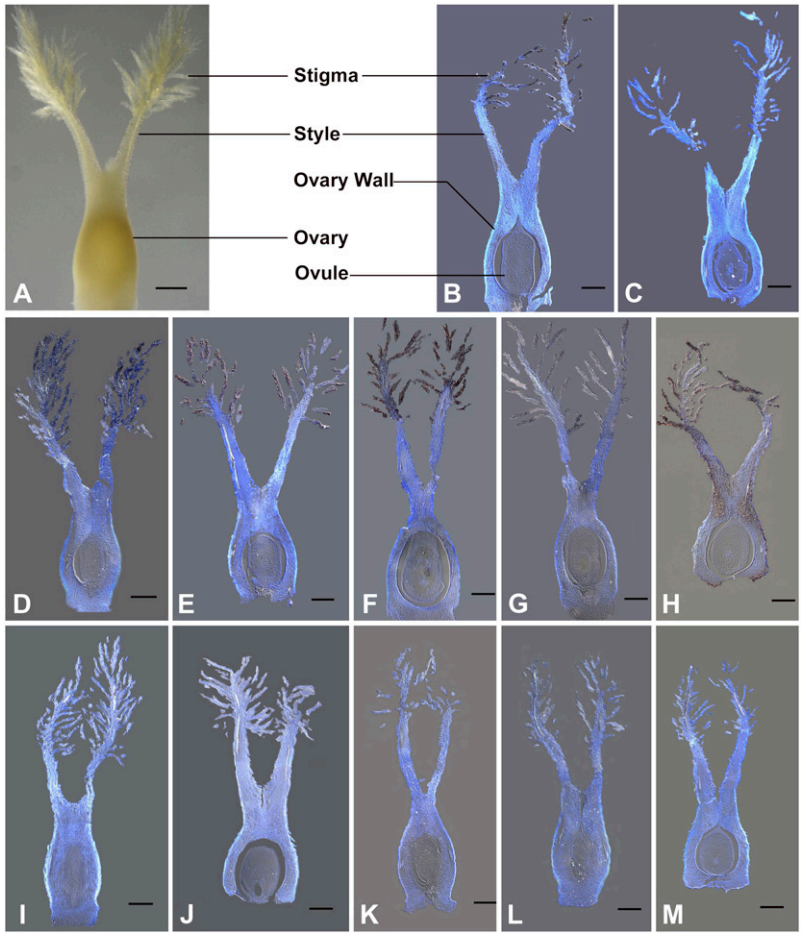

Figure 1. Expression of five stigma preferential genes by RNA in situ hybridization. A, A rice pistil before pollination. B, D, E, F, G, and H, Hybridization by the antisense probes of LOC_Os02g46260, LOC_Os09g36700, LOC_Os10g33250, LOC_Os01g62070, AK071040, and LOC_Os01g09620, respectively. C, I, J, K, L, and M, Hybridization by their respective sense probes. Among them, the gene LOC_Os01g09620 (H and M) was used as a reference, because the microarray result showed that it was expressed in the entire mature pistil. The longitudinal section through the pistil before pollination was used for all the hybridizations. All of the slices were stained with Fluorescent Brightener 28 after hybridization. The hybridization signals are shown in dark purple. The structures of the pistil are illustrated in A and B. Bar = 100 μm.

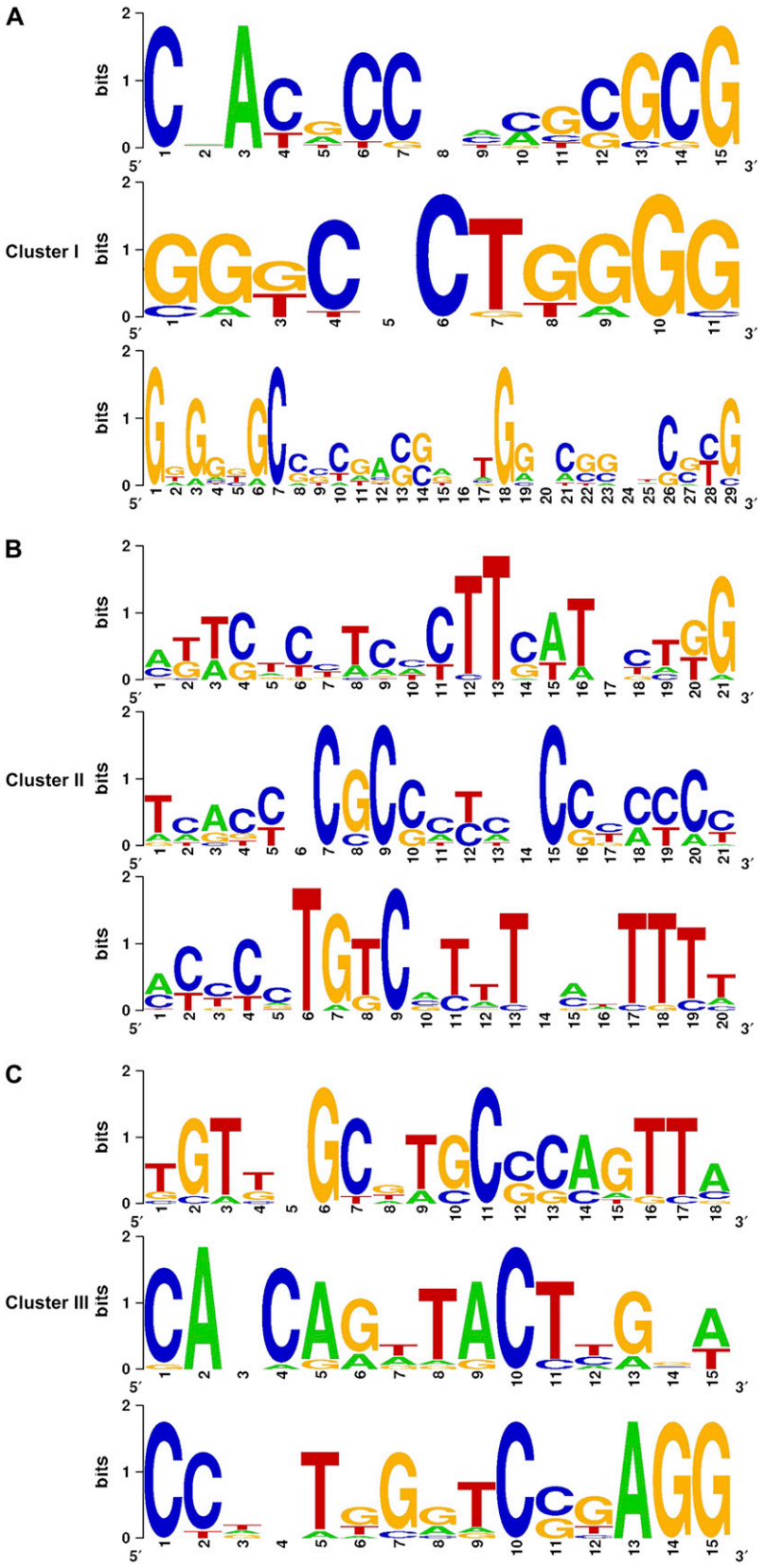

Figure 2. Putative cis-acting regulatory elements enriched in the stigma-specific or preferential genes. A to C, The top three candidate motifs of cluster I, cluster II, and cluster III, respectively. The overall height of each stack indicates the sequence conservation at that position (measured in bits), whereas the heights of symbols within the stack reflect the relative frequencies of the corresponding nucleic acids at that position.

GCC box (Brown et al., 2003; Chakravarthy et al., 2003). Most of genes in cluster III had Myb-transcription factor recognition sites that are usually found in the promoters of the dehydration-responsive genes. One of the motifs (Fig. 2C, bottom) in cluster III appeared to have unknown function, indicating that they might represent new cis-acting elements in regulating the transcription of the stigma-specific genes.

DISCUSSION

In this study, we have identified 548 genes expressed specifically or predominantly in the stigma papillar cells of rice by using 57 K Affymetrix rice whole-genome array and 10 K rice cDNA microarray. It is highly likely that they represent good candidates for the stigma-specific genes in rice. First, for the two different technological platforms we used, there existed good correlations, and the variables observed were reasonable according to several recent reports (Maruyama et al., 2004; Yauk et al., 2004; Hannah et al., 2005). Second, the qRT-PCR and RNA in situ hybridization confirmed the microarray results (Table II; Fig. 1). Third, the rice stigma gene data set is similar to that identified in Arabidopsis (Tung et al., 2005). Thus, the careful selection of the starting tissues is an effective approach to identify the stigma-specific genes using the whole-genome and cDNA microarrays.

The Stigma-Specific Genes Have Conservative Roles in Plants

The functional annotation of both rice and Arabidopsis genes specifically or preferentially expressed in stigma suggests that several groups of the genes appear to play conserved roles in the stigma. Forty-two Arabidopsis stigma-specific genes representing 36% of the gene set are highly similar to 83 genes representing about 15% of the rice stigma gene dataset (Supplemental Table S8). The majority of them belong to the cell wall-related and signal transduction groups, indicating that these two classes of genes have conserved functions in the stigma. Swanson et al. (2005) also found that these two functional categories were overrepresented in contrast to the distributions of the total stigma message RNA. The cell wall-related group represents the second largest group of the genes identified in the rice stigma gene data set, which include the genes encoding pectinesterase, peroxidase, and α-expansin. They are putative cell wall-localized enzymes and may be the most suitable candidates for the papilla cell wall loosening or expansion (Wu et al., 1996; Schopfer, 2001). The cellulose synthase, glycosyl transferase, and endoglucanase may be involved in the cell wall synthesis required for the pollen tube growth (Hong et al., 2001; Goubet et al., 2003; Aspeborg et al., 2005; Yokoyama and Nishitani, 2006). Consistent with the cell-cell communication in the pollen-pistil interaction, the rice stigma gene dataset also contained a significant proportion of signal transduction-related genes (8.9%). Protein kinases play crucial roles in a wide variety of cellular functions, including proliferation and differentiation, by adding phosphate groups to Thr, Tyr, and Ser residues of specific target proteins. The plant receptor-like kinases likely are transmembrane proteins that transduce external messages into the cell through their extracellular domains and intracellular kinase domains. Previously, this protein category was identified and implicated in the pollen-pistil interaction and pollen tube growth (Muschietti et al., 1998; Tang et al., 2002). In *Brassica*, the stigma-expressed SRK interacts with SCR and initiates a signal transduction cascade that inhibits the pollen rehydration and, consequently, the growth of self pollen, while allowing nonself pollen to grow (Kachroo et al., 2002; Takayama and Isogai, 2005). The putative protein kinases and putative protein receptor-like kinase identified in the rice stigma gene sets are likely involved in the pollen-stigma interaction and the early steps of pollination. The three genes confirmed by whole mount RNA in situ hybridizations in the Arabidopsis study all had the homologous genes in our stigma-specific gene list (Tung et al., 2005). At5g59810 is related to LOC_Os02g44590, encoding an expressed subtilisin-like protease precursor protein; At5g19880 is related to LOC_Os06g48030, encoding an expressed putative peroxidase 16 precursor; and At2g02850 is related to LOC_Os03g02400, encoding an expressed early nodulin-like protein 1 precursor protein. Thus, our results and those using Arabidopsis suggest that the stigma involves the genes with the highly conserved functions.

The Phytohormone Auxin and Stigma Function during Pollination

Auxin is the central growth regulator of a myriad of aspects of plant growth and developmental processes and appears to be actively transported throughout the plant to control cell division, extension, and differentiation (Benjamins et al., 2005). Several roles have been suggested for auxin in the pollination process. In orchid, it has been shown that the pollination and auxin regulate the ethylene production and ovary development, because when inhibitors of ethylene were used, the pollination- or auxin-induced ovary development was inhibited (Zhang and O'Neill, 1993). During Arabidopsis flower development, the concentration of free auxin increased gradually, starting at the floral organ tip visualized by immunolocalization with polyclonal antibodies against auxin and accumulated in pollen grains and stigma before fertilization (Aloni et al., 2006). In maize, the augmentation of auxin in the pistil tissues is important for egg cell differentiation (Mol et al., 2004).

However, it is unclear whether auxin is involved directly in the interaction of the pollen and stigma. In the hormone-related genes group from our data set, the auxin-related genes appear to be overrepresented

(Table I; Supplemental Table S7), and the results of the GO analysis of the rice stigma also suggested that the auxin signaling plays a significant role in the rice stigma function (Supplemental Fig. S1). The five of the identified auxin-related genes are *SAUR*s (Jain et al., 2006). They are LOC_Os04g56690, LOC_Os09g37430, LOC_Os09g37440, LOC_Os09g37490, and LOC_Os09g37500, namely *OsSAUR23*, *OsSAUR48*, *OsSAUR49*, *OsSAUR54*, and *OsSAUR55*, respectively. Interestingly, four of these belong to a big gene cluster that contains 17 *OsSAURs* contiguously arranged in chromosome 9 (Jain et al., 2006). The *SAURs* encode short-lived nuclear proteins that are induced within minutes after auxin application and may play a role in auxin-mediated cell elongation (McClure and Guilfoyle, 1989; Franco et al., 1990; Gee et al., 1991; Knauss et al., 2003). It was reported that a suitable amount of auxin could lead to loosening of the cell wall and promote cell growth through inducing H(+) secretion and activating cellulose synthesis (Fry et al., 1990). The auxin response factor seems to control the expression of the *OsSAUR*s through the auxin-responsive elements present in their promoter regions (Ulmasov et al., 1997). These studies indicate that auxin may be involved in loosening the stigma papillar cell wall and promoting pollen tube growth through inducing the expression of *OsSAUR*s. The IAA-amido synthetase *GH3.1* gene (LOC_Os01g57610) is involved in the auxin homeostasis through the conjugation of IAA to amino acids (Terol et al., 2006). But it is unclear where the auxin comes from. Could it be synthesized in the stigma papillar cell or transported from other organs (see below)? Further analyses of these candidate genes will shed light on this aspect of auxin signaling in the stigma during pollination.

Transport Function in the Stigma

The transport-related proteins are mostly abundant in the rice stigma gene dataset and appear to be actively involved in the stigma function. The rice pollen is not fully dehydrated and metabolically active when shed from the anthers. They likely require the stigma to be fully developed to supply all the nutrition and metabolites for pollen germination and tube growth. The different kinds of transporters appear to be involved in the exchange of materials and information between pollen and stigma. The ABC transporter is one of the active transport systems of the cell, which is widespread in prokaryotes and eukaryotes. In tobacco, the gene *NtWBC1* is developmentally regulated in the stigma/style, with mRNA accumulation increasing toward anthesis (Otsu et al., 2004). RNA in situ hybridization experiments demonstrated that *NtWBC1* is predominately expressed in the stigmatic secretory zone. There are four ABC transporters expressed in the stigma papillar cells of rice (Table I; Supplemental Table S7). Two recent reports indicate that plant ABC transporters mediate the cellular and long-distance transport of the plant hormone auxin (Sidler et al., 1998; Multani et al., 2003). The functional study of the four ABC transporters may help to clarify the mechanism of the auxin signaling in the stigma during pollination. The potassium uptake gene expression was highly enriched in the stigma. Recently, it has been reported that potassium may regulate anther dehiscence, pollen imbibition, and papilla hydration (Rehman and Yun, 2006). In *Brassica*, aquaporin-like proteins in the stigma may act as water channels to regulate water flow into the pollen grain from the stigma (Dixit et al., 2001). There are 33 genes annotated for aquaporins in the rice genome that are predicted to enable the fast and controlled translocation of water across the membrane, and most of them have been identified and characterized recently (Sakurai et al., 2005; Guo et al., 2006). From our dataset, one aquaporin protein-encoding gene, *PIP2.2* (LOC_Os02g41860), was identified to have a specific and enriched expression in the rice stigma papilla cells, suggesting that it may function in the stigma during pollination. However, it remains an important task to address their roles in pollination.

Possible Cross Talk of Stress Responses and Pollination

Previously, we detected an extensive overlap of the genes involved in pollination and abiotic stress responses (Lan et al., 2005). Consistent with this, many stigma-specific genes also were related to stress and defense genes, such as those encoding MLO-like proteins, heat shock protein-binding proteins, disease resistance proteins, Bowman-Birk-type bran trypsin inhibitor precursor proteins, and wound-induced protein WI12-containing protein (Table I; Supplemental Table S7). Defense-related compounds have been found to accumulate specifically in the stigma; the proteinase inhibitor proteins and chitinase are highly enriched in stigmas of *Solanaceous* plants (Leung, 1992; Atkinson et al., 1993). It has been shown that the pollination and wounding induced nearly identical flavonol kinetics in petunia and patterns of the accumulation in the outer cell layers and exudates of the stigma, suggesting that they share elements of a common signal transduction pathway (Vogt et al., 1994). In petunia flowers, both pollination and stigma wounding induced a transient increase in ethylene production and hastened corolla senescence (Woltering et al., 1997). Further, it has been reported that the *SPP2* (*Solanum pollinated pistil*) dioxygenase from self-incompatible wild potato (*Solanum chacoense*) is predominantly expressed in the pistil and could be induced by wounding of the style as well as pollination/fertilization (Lantin et al., 1999). The SPP2 dioxygenase could be involved in the biosynthesis of deterrent alkaloids in reproductive tissues or in generating chemical signals involved in pollen tube guidance (Lantin et al., 1999). One lectin domain-encoding gene belonged to the stress-related group. A b-lectin receptor kinase gene has been shown to confer the rice blast resistance (Chen et al., 2006). Recently, Chu et al. (2006) have demonstrated that *R* gene *xa13*, a recessive allele conferring disease resistance against bacterial blight, plays a key role in both disease resistance

and pollen development and a trade-off exists between fertility and pathogen defense, depending on the levels of expression of the *xa13* gene.

Our analysis also revealed that the stigma-specific genes share some common cis-regulatory elements with the stress-responsive genes. A large percentage of genes in cluster I possess the same motif, named GCC box (Fig. 2). The GCC box has been found in many pathogen-responsive genes (Brown et al., 2003; Chakravarthy et al., 2003). GbERF belongs to the ethylene-responsive factor (ERF) family of transcription factors and regulates the GCC box containing pathogen-related genes (Qin et al., 2006). The GCC box is found not only in the pathogen-responsive genes but also existed in some abiotic stress-related genes. In Arabidopsis, AtERF7 binds to the GCC box, and overexpressing *AtERF7* showed a reduced sensitivity to drought stress (Song et al., 2005). The rice OsBIERF3 could bind specifically to the GCC box sequence and was induced by salt, cold, drought, wounding, and the rice blast fungus (Cao et al., 2006). Further work is required to dissect the molecular basis of this possible cross talk.

In conclusion, we have identified a large set of genes that are specifically or highly enriched in the rice stigma. In addition to the conserved roles of the cell wall metabolism and cellular communication in the stigma, the identification of genes involved in the auxin signaling, transcription functions, and possible cross talk between pollination and stress/defense responses provides new insights into the molecular functions of the stigma in rice.

MATERIALS AND METHODS

Plant Materials

Rice (*Oryza sativa*) 'Nipponbare' seeds were germinated and grown in a growth container for 2 weeks (28°C, 16 h light, 8 h dark, and photo intensity of 240 μm photos m^{-2} s^{-1}). Then, shoot and root were harvested for Sh and Rt, respectively. Other plant materials used in this study described below were harvested from rice plants grown under natural conditions in the field of the Institute of Genetics and Developmental Biology, Beijing, during May to October and Hainan, China, during December to April. An were collected at a stage 1 to 2 d before floret flowering; unpollinated St and Ov were dissected at a stage 0 to 1 d before floret flowering; pistils of 5DAP were dissected 5 d after anthesis, 10EM and 10EN were dissected from grains 10 d after flowering, respectively.

Suspension Cell Culture

The method used for suspension cell culture was described by Wang et al. (2005).

10 K cDNA Microarray Hybridization and Data Analysis

Total RNA was isolated using RNeasy kit (Qiagen). Double-strand cDNA was synthesized from 5 μg total RNA using cDNA Synthesis kit (TaKaRa). In vitro transcription from cDNA to cRNA was performed using T_7 RiboMAX Express Large Scale RNA Production system (Promega). Then the cRNA was converted to DNA by using Superscript II RT kit (Invitrogen) and random primers. One microgram DNA product and random primers (9-mers) were annealed to denatured DNA template and extended by Klenow fragment (TaKaRa) in the presence of Cy5-dCTP/Cy3-dCTP (Amersham Pharmacia) for target preparation in microarray analysis. Hybridization and washing were performed as described in CyScribe Post-Labeling kit (Amersham Pharmacia) and CMTTM hybridization chamber (Corning) user manuals. Scanning and data acquisition were performed on a GenePix 4000B scanner using GENEPIX 4.0 software (Axon Instruments). GenePix Pro 4.0 output files were converted to The Institute for Genomic Research Multi Experiment Viewer file (.mev) by ExpressConverter V1.4 (http://www.tigr.org/software/tm4/utilities.html) and normalized (local lowess) by MicroArray Informatics Discovery System (http://www.tigr.org/software/tm4/). Then, spots flagged bad or not found by Genepix software were removed from further data analysis, and only those spots that showed fluorescent intensity levels in at least one channel above the background (local) + 2SD were used for further analysis. Those spots that exhibited a large difference between the duplicate experiments (dye swap) were regarded as outliers and removed manually. Hierarchical clustering was performed as described by Eisen et al. (1998).

Affymetrix GeneChip Hybridization and Data Analysis

Total RNA was isolated using TRIzol reagent (Invitrogen) and purified by using Qiagen RNeasy columns (Qiagen). For Affymetrix GeneChip (Affymetrix) analysis, 8 μg total RNA was used for making biotin-labeled cRNA targets. All the processes for cDNA and cRNA synthesis, cRNA fragmentation, hybridization, washing and staining, and scanning were conducted according to the GeneChip standard protocol (Eukaryotic Target Preparation, Affymetrix). Poly-A RNA Control kit and the One-Cycle cDNA Synthesis kit were used in this experiment as described in the Web site: http://www.affymetrix.com/products/arrays/specific/rice.affx. The information about GeneChip Rice Genome Array (MAS 5.0) could be accessed from the Affymetrix Web site: http://www.affymetrix.com/products/arrays/specific/rice.affx. Affymetrix GeneChip Operating software (GCOS) was used for data collection and normalization. The overall intensity of all probe sets of each array was scaled to 500 to guarantee that hybridization intensity of all arrays was equivalent, each probe set was assigned with P, A, and M and a *P* value from the algorithm in GCOS. To identify differentially expressed genes, the $\log_2$ transformed signal ratio of each gene was calculated using the GCOS baseline tool, and $\log_2$ (ratio) $\geq$ 1.33 (2.5-fold change) was used as cutoff.

Two statistical methods were used for data processing. First, analysis using the Significance Analysis of Microarrays software package was conducted for rice triplicate samples between stigma and ovary using q value $\leq$ 0.05 and fold-change $\geq$2-fold change as cutoff, and 7,425 probe sets were subsequently picked out and their expression levels were significantly different between stigma and ovary. We also did normal *t* test for each genes and added a *P* value for them. Second, we used the *Z*-score transformation normalization method to compare expression levels from stigma and other organs or tissues and suspension-cultured cells and directly calculated significant changes in gene expression between different samples. *Z* scores were calculated by taking the difference between the averages of stigma (X_i) and other test samples (μ, mean) and dividing by the SD of all the other samples using the following equation:

$$Zi = \frac{Xi - \mu}{\mathrm{SD}}$$

In total, we identified 665 probe sets preferentially expressed in stigma using *Z* score $\geq$ 2.32 and *P* value $\leq$ 0.01. For *Z*-score transformation normalization, we used the average value from stigma and ovary separately. The test samples included Sh, Rt, An, 5DAP, 10EM, 10EN, and suspension-cultured cell. In addition, we measured the relative ratio between stigma and other samples using the following equation:

$$\mathrm{Ratio} = \frac{\overline{S}}{Max(\overline{O}, T1, T2, \ldots, Tn)}$$

$\overline{S}$ is the average of expression level in stigma tissue sample; $\overline{O}$ is the average of expression level in the ovary tissue sample; and *T1*, *T2*, …,*Tn* are the expression levels in other tissue samples. The ratios of all 665 probe sets were more than a 2-fold change.

GO Analysis

We searched GO information for the 665 probe sets using EasyGO software (http://bioinformatics.cau.edu.cn/easygo/category_treeBrowse.html). Using the rice 'Japonica' gene database, we applied χ^2 tests for the biological process search, and the cutoff for false discovery rate was adjusted and the *P* value was 0.0001.

Real-Time PCR

Total RNA preparation and real-time PCR were performed as previously described (Lan et al., 2004). In brief, 2 μg total RNA was used for cDNA synthesis with SuperScript III First-Strand Synthesis kit (Invitrogen). The cDNA samples were diluted to 8 and 2 ng/μL. Triplicate quantitative assays were performed on 1 μL of each cDNA dilution using the SYBR Green Master mix (Applied Biosystems) with an ABI 7900 sequence detection system according to the manufacturer's protocol (Applied Biosystems). Gene-specific primers were designed by using PRIMEREXPRESS software (Applied Biosystems). The relative quantification method (delta-delta threshold cycle) was used to evaluate quantitative variation between replicates examined. Amplification of 18S rRNA was used as an internal control to normalize all data.

RNA in Situ Hybridization

RNA in situ hybridization was performed as previously described (Lai et al., 2002). Mature rice pistils at the stage 0 to 1 d before floret flowering were fixed with formalin-acetic acid-alcohol fixative solution at 4°C overnight followed by dehydration steps and then embedded in paraffin (Paraplast Plus; Sigma). The tissues were sliced into 8-μm sections with a microtome (Leica RM2145; Leica Microsystems) and affixed to Poly-Prep slides (Sigma). The tissues were stained with Fluorescent Brightener 28 (Sigma) after hybridization. Images were observed under bright and fluorescence field at the same time through a microscope (Olympus BX51; Olympus Optical) and photographed using a Micro Color CCD camera (Apogee Instruments).

Promoter Analysis

The 1,000-bp regions located upstream of the start codons of genes of interest were used for analysis with the MEME/MAST system (http://meme.sdsc.edu/meme/meme.html) and PLACE (http://www.dna.affrc.go.jp/PLACE). Sequence logos of cis-acting regulatory elements were created by weblogo (http://weblogo.berkeley.edu/logo.cgi).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. GO term enrichment status for the putative rice stigma-specific genes.

Supplemental Figure S2. Hierarchical clustering of the stigma-preferential genes from the three clusters with the eight tested organs or tissues and suspension culture cells.

Supplemental Table S1. Significantly expressed probe sets of stigma compared to ovary identified by 57 K Affymetrix rice whole-genome array.

Supplemental Table S2. Candidate cDNA clones highly expressed in stigma identified by 10 K cDNA microarray.

Supplemental Table S3. Candidate cDNA clones highly expressed in ovary identified by 10 K cDNA microarray.

Supplemental Table S4. Candidate genes highly expressed in stigma identified by both the two microarrays.

Supplemental Table S5. Candidate genes highly expressed in ovary identified by both the two microarrays.

Supplemental Table S6. Candidate probe sets highly expressed in stigma identified by 57 K Affymetrix rice whole-genome array.

Supplemental Table S7. Classifications of candidate genes highly expressed in stigma identified by 57 K Affymetrix rice whole-genome array.

Supplemental Table S8. Candidate stigma-specific genes conserved in rice and Arabidopsis.

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