Supplementary data to:

Zm908p11, encoded by a short open reading frame (sORF) gene, functions in pollen tube growth as a profilin ligand in maize

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Supplementary material and methods section

Constructs for tobacco transformation

35S-Zm908s and *35S-Zm908a*

The full length of *Zm908* cDNA was amplified from genomic clone *Zm908* with primer pair 9pf and 9pr (Table S1) on a S1000™ thermal cycler (Bio-Rad, USA). The PCR product, named *Zm908s,* was cloned into pMD18-T (TaKaRa, Japan) to produce pMD18-Zm908s. *Zm908s* was then excised with *Sac*I and *Xba*I from pMD18-Zm908s and inserted into the same sites of pBI121 between the *CaMV35S* promoter, and the *Nos* terminator, replacing the *GUS* gene to generate the sense construct *35S-Zm908s* (Fig. S1A). *Zm908s* was first excised by *Hin*dIII from pMD18-Zm908s, end filled with dNTP using Klenow (TaKaRa. Japan), then excised by *Sac*I and inserted into the *Sac*I and *Sma*I sites of pBI121 between the *CaMV35S* promoter, and the *Nos* terminator, replacing the *GUS* gene to generate the antisense construct *35S-Zm908a* (Fig. S1B).

35S-Zm908p11, *35S-Zm908p11ss* and *35S-Zm908p11fs*

The longest ORF (+697 to +990) was amplified from pMD18-Zm908s with primer pair 9p11f and 9p11r (Table S1). The PCR product, called *Zm908p11*, was cloned into the *Bam*HI and *Sac*I sites of pBI121 to produce *35S-Zm908p11* (Fig. S1C). The primer pairs 9pf/9pr, 9p11ssf/9p11ssr and 9p11fsf/ 9p11fsr (Table S1) were used to generate a site-specific mutation (the ATG start codon was replaced by AAG), and a frame-shift mutation (the ATG start codon was replaced by ATGA) of *Zm908p11* from pMD18-Zm908s by overlapping PCR. After sequencing, the fragments *Zm908p11ss* and *Zm908p11fs* were excised using *Bam*HI and *Sac*I, and cloned into pBI121 between the *CaMV35S* promoter, and the *Nos* terminator, replacing the *GUS* gene to produce the constructs *35S- Zm908p11ss* (Fig. S1D); and *35S-Zm908p11fs* (Fig. S1E).

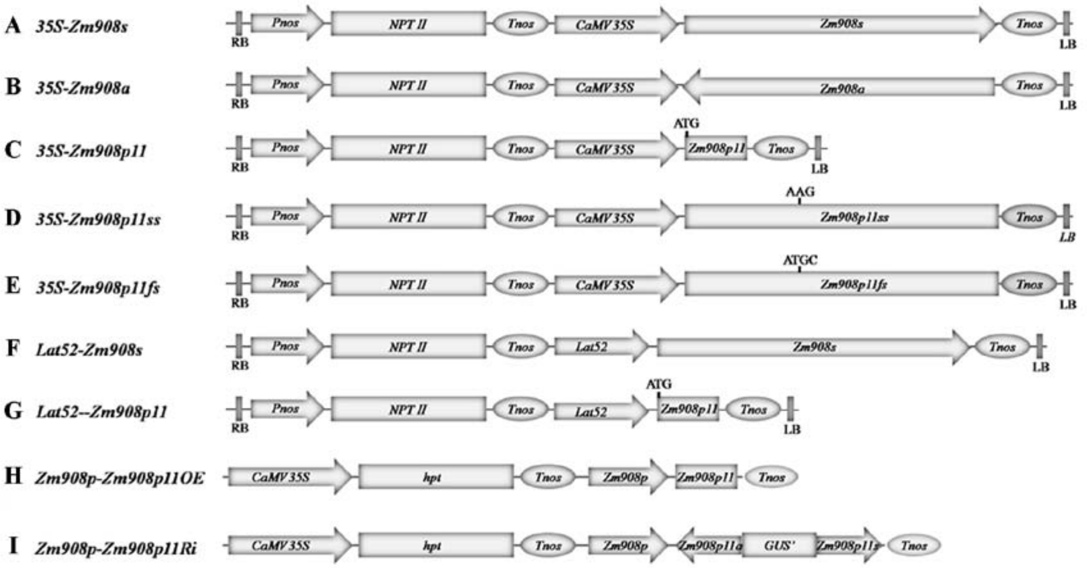
Lat52-Zm908s and *Lat52-Zm908p11*

The *Lat52* promoter was amplified from tobacco pollen cDNA with primer pair lat52f and lat52r (Table S1), and inserted into the *Hin*dIII and *Bam*HI sites of *35S-Zm908s* and

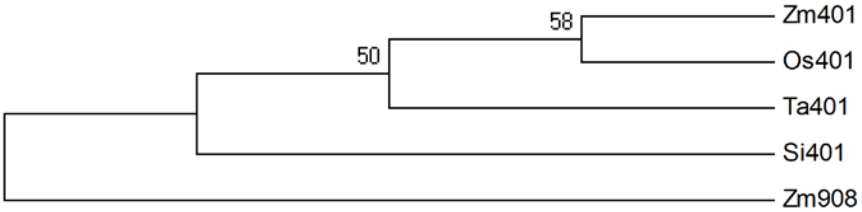
35S-Zm908p11, replacing the *CaMV35S* promoter to produce *Lat52-Zm908s* (Fig. S1F); and *Lat52-Zm908p11* (Fig. S1G).

Constructs for maize transformation

A 282 bp promoter fragment of *Zm908* (*Zm908p*) was amplified from genome clone *Zm908* with primer pair 9prof and 9pror (Table S1), and then cloned into the *Hin*dIII and *Bam*HI sites of pMD18-T to produce pMD18-Zm908p. The *Zm908p* fragment was excised with *Hin*dIII and *Bam*HI from pMD18-Zm908p, and inserted into the same sites of pROK219 to generate pROK219-Zm908p. The *Zm908p11* fragment was excised from *35S-Zm908p11* with *Bam*HI and *Sac*I and inserted into the corresponding sites of pROK219-Zm908p, and then fused with an *hpt* gene (hygromycin B phosphotransferase) controlled by the *CaMV35S* promoter at *Hin*dIII site to produce the overexpression construct *Zm908p-Zm908p11OE* (Fig. S1H). To generate the RNAi construct, the 3'-UTR of *Zm908* was amplified from pMD18-Zm908s with primer pairs 9-3af/9-3ar and 9-3sf/9-3sr (Table S1), and cloned into the *Bam*HI/*Xba*I and *Nco*I/*Sac*I sites of pGEX-KG respectively, to generate pGEX-KG-stem. The loop fragment was amplified from the *GUS* gene of pBI121 with primer pair gusf and gusr (Table S1), and inserted into the *Xba*I and *Nco*I sites of pGEX-KG-stem. The stem-loop fragment was excised using *Bam*HI and *Sac*I, and cloned into the same sites of pROK219-Zm908p. Finally, an *hpt* gene controlled by the *CaMV35S* promoter was fused with pROK219-Zm908p-stemloop at *Hin*dIII site to produce *Zm908p-Zm908p11Ri* (Fig. S1I).

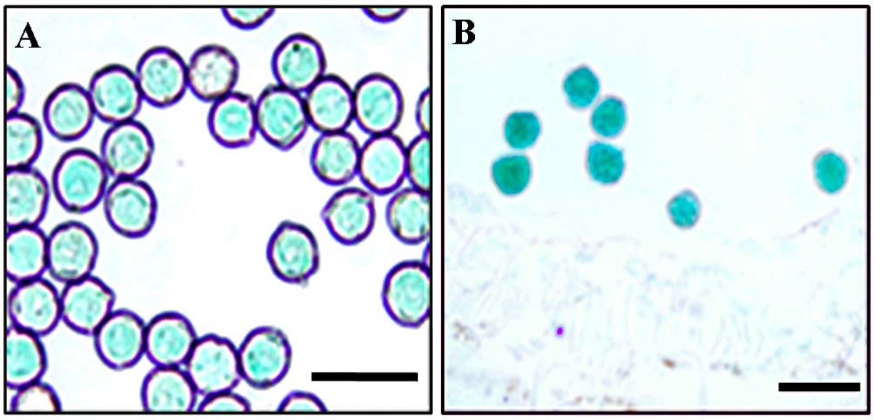


Supplemental Fig. S1: Diagram of the constructs used in the present study. RB, right border; LB, left border; *Pnos*: nopaline synthase gene promoter; *NPTII*, gene encoding neomycin phosphotransferase II; *Tnos*: nopaline synthase gene terminator; *CaMV 35S*, *cauliflower mosaic virus 35S* promoter; *Zm908p11*, the longest ORF of *Zm908* cDNA; *Zm908p11ss*, *Zm908* cDNA with a site-specific mutation in the ATG start codon of *Zm908p11*; *Zm908p11fs*, *Zm908* cDNA with a frame-shift mutant of *Zm908p11*; *hpt*, hygromycin B phosphotransferase gene; *Zm908p11a*, antisense of 3'-UTR of *Zm908; GUS'*, a part of *GUS* gene; *Zm908p11s*, sense of 3'-UTR of *Zm908.*

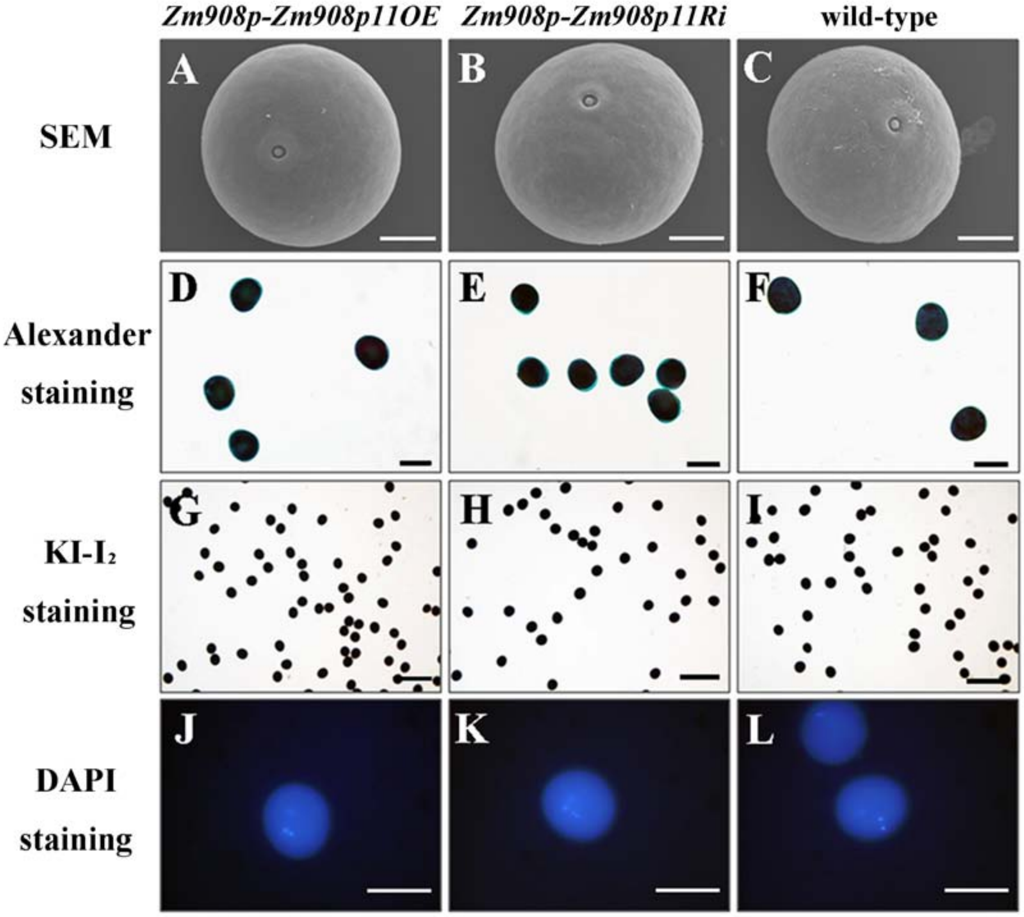


Supplemental Fig. S2: Phylogenetic analysis of *Zm908.*

Zm401, *Zea mays* AY911609; *Os401*, *Oryza sativa* EU426833; *Ta401*, *Triticum aestivum* EU426832; *Si401*, *Setaria italica* DQ981487.



Supplemental Fig. S3: GUS staining of pollen grains and anther from *Zm908p-GUS* transgenic tobacco. (A) GUS staining of pollen grains from *Zm908p-GUS* transgenic anther. (B) Cross sections of the anther of *Zm908p-GUS* transgenic tobacco. Bars = 100 µm.



Supplemental Fig. S4 Phenotypic analysis of pollen from *Zm908-Zm908p11OE, Zm908p-Zm908p11Ri* and wild-type maize plants.

(A), (D), (G) and (J) Pollen grains from *Zm908p-Zm908p11OE* plants. (B), (E), (H) and (K) Pollen grains from *Zm908p-Zm908p11Ri* plants. (C), (F), (I) and (L) Pollen grains from wild-type plants. (A) to (C) Scanning electron microscopy (SEM). Bars = 20 μm. (D) to (F) Alexander staining. Bars = 50 μm. (G) to (I) KI-I_2 staining. Bars = 200 μm. (J) to (L) DAPI staining. Bars = 50 μm.

Supplemental Table S1: List of primers used for gene cloning and qRT-PCR assays

Primer name	Primer sequence
9pf	5’-CGCTCGCACTAGAGAACTTG-3’
9pr	5’-CAAGAGATATTCGGGCATAA-3’
9p11f	5’-CGGGATCCTCACATGACCTACAATGAGG-3’
9p11r	5’-CGGAGCTCCGAAGCTCTTCAAATCGCT-3’
9p11ssf	5’-TGACCTACAAAGAGGTGGTAC-3’
9p11ssr	5’-ATGTACCACCTCTTTGTAGGT-3’
9p11fsf	5’-TGACTTACAATGAAGGTGGTAC-3’
9p11fsr	5’-ATGTACCACCTTCATTGTAGGT-3’
9prof	5’-TCAAGCTTGTATTTAATAAAAATAGTTCCAAGATTTT-3’
9pror	5’-GGGGATCCAATGGTCAGAGAAACATATGAATAG-3’
9-3af	5’-GCTCTAGAGCCCCTCTATTTCTGTTTCTTC-3’
9-3ar	5’-CGCGGATCCGCGTACTTCTCTGGTTTGTGTTCTA-3’
9-3sf	5’-CATGCCATGGCATGCCCTCTATTTCTGTTTCTTC-3’
9-3sr	5’-CGAGCTCGTACTTCTCTGGTTTGTGTTCTA-3’
gusf	5’-GCTCTAGAGCTTACGGCAAAGTGTGGGTC-3’
gusr	5’-CATGCCATGGCATGTTGCCAGAGGTGCGGATT-3’
prtf	5’-TACAATGAGGTGGTACATGGCT-3’
prtr	5’-TCAAATCGCTCTATCAGATGGAC-3’
trtf	5’-GAGCATGGCATTCAGGCTCACG-3’
trtr	5’-TCAACAAAAACAGCACGGGGCA-3’
9Gf	5’-GATATCATGAGGTGGTACATGGCT-3’
9Gr	5’-GGTACCAATCGCTCTATCAGATGGACT-3’
y2h9Nf	5’-CGGGATCCCGATGAGGTGGTACATGGC-3’
y2h9Nr	5’-GTCGACGAATCGCTCTATCAGATGGACT-3’
y2hPf	5’-CCGGAATTCATGTCGTGGCAGACGT-3’
y2hPr	5’-CTCGAGCTTACATCCCCTG-3’
L9f	5’-GGGGTACCATGAGGTGGTACATGG-3’
NL9r	5’-ACGCGTCGACAATCGCTCTATC-3’
CL9r	5’-ACGCGTCGACTCAAATCGCTCTATC-3’
LPf	5’-GGGGTACCATGTCGTGGCAGACGT-3’
NLPr	5’-ACGCGTCGACTTACATCCCCTG-3’
CLPr	5’-ACGCGTCGACTCATTACATCCCCTG-3’

Supplemental Table S2: List of genes upregulated and downregulated which were changed more than 2-fold in the microarray analysis

Affymetrix number	Fold change (OE/WT)	GenBank ID	Putative function
Zm.12272.1.A1_at	153.77	BM351641	---
Zm.3837.1.S1_at	144.27	AI978033	epoxide hydrolase 2
Zm.10168.1.A1_at	123.05	CA404699	---
Zm.18100.4.A1_at	101.62	CD999582	ACT domain containing protein
Zm.934.1.S1_at	51.484	BF729446	---
Zm.9879.1.A1_at	33.563	BM339505	hypothetical protein LOC100217169
Zm.3561.1.A1_at	31.929	CK826673	hypothetical protein LOC100275649
Zm.8749.1.A1_at	27.078	BM335905	---
Zm.18100.1.S1_a_at	17.995	AI967288	---
Zm.2571.1.A1_at	14.344	AY106387.1	---
Zm.12367.1.S1_at	12.932	BQ577550	---
Zm.241.1.S1_at	12.859	CK786973	Superoxide dismutase4
Zm.2787.1.A1_at	9.593	AY105546.1	hypothetical protein LOC100191148
Zm.6364.1.A1_at	9.354	AI691566	hypothetical protein LOC100277739
Zm.1798.1.A1_a_at	8.716	BE519060	---
ZmAffx.447.1.A1_at	8.195	AI677073	hypothetical protein LOC100193673
Zm.13280.1.S1_at	8.106	CA826511	---
Zm.12666.1.S1_a_at	7.411	AY536122.1	meg2 protein/meg4 protein/meg6 protein
Zm.2077.1.A1_at	7.202	CA401374	---
Zm.18006.1.A1_at	7.076	AW400144	hypothetical protein LOC100273180
Zm.10809.1.A1_a_at	6.863	AY104721.1	mitochondrial inner membrane protease subunit 1
Zm.16504.1.A1_at	6.483	X54076.1	heat shock protein18f
Zm.13137.2.S1_a_at	6.235	CO518420	---
Zm.12259.1.S1_at	5.633	BQ578011	---
Zm.12557.1.A1_at	5.592	AI691470	---
Zm.11746.1.A1_at	5.533	BM380405	---

Zm.7156.3.S1_a_at	5.373	CF017430	hypothetical protein LOC100193362
Zm.6431.1.A1_at	5.223	CO525297	hypothetical protein LOC100193964
Zm.6448.1.A1_at	5.054	BG842262	antigenic determinant of rec-A protein
Zm.18319.1.A1_at	5.024	CO531171	hypothetical protein LOC100216754
Zm.10461.1.S1_at	4.991	CA826724	---
Zm.10498.1.S1_at	4.979	BQ528852	---
Zm.16813.1.S1_at	4.564	AI649555	---
Zm.15840.1.A1_at	4.502	CK827017	receptor kinase 1
Zm.15965.1.A1_at	4.441	AI677363	signal peptidase complex subunit 1
Zm.13302.1.S1_at	4.435	CA827293	---
Zm.12666.1.S1_x_at	4.424	AY536122.1	meg2 protein
Zm.6315.1.A1_at	4.309	AI691364	---
Zm.4049.1.A1_at	4.214	AI834098	hypothetical protein LOC100279705
Zm.3782.1.S1_at	4.204	AW066955	vacuolar protein sorting 26
ZmAffx.1425.1.S1_at	4.127	11990232-10	---
Zm.12666.4.S1_x_at	4.109	AY536123.1	meg2 protein/meg6 protein
Zm.2922.1.A1_at	4.031	CO532813	---
Zm.15937.1.A1_at	4	CO520791	---
Zm.4256.1.S1_at	3.954	CA403577	---
Zm.16468.1.S1_at	3.904	CD999619	hypothetical protein LOC100277908
Zm.17.1.A1_at	3.876	M76684.1	orange pericarp1
Zm.17300.1.A1_a_at	3.869	CK371224	hypothetical protein LOC100275883
Zm.17428.1.A1_at	3.851	CK145261	pyridoxin biosynthesis protein ER1
Zm.1581.1.S1_at	3.816	AW566202	---
Zm.5653.1.A1_at	3.642	BM074926	---
ZmAffx.1509.1.S1_at	3.637	11990232-51	---
ZmAffx.1468.1.S1_at	3.632	11990232-35	---
Zm.8651.1.A1_at	3.551	BM075068	hypothetical protein LOC100275908
Zm.17694.1.S1_at	3.534	CK370322	---

Zm.10069.1.A1_at	3.519	AY108373.1	---
Zm.1974.1.A1_at	3.301	CF920129	---
Zm.6485.1.A1_at	3.292	CB617134	growth-regulating factor 2-like
Zm.4040.1.A1_at	3.282	AI834032	---
Zm.6961.1.A1_at	3.281	AY104000.1	hypothetical protein LOC100191822
Zm.13394.1.A1_at	3.275	AW066700	---
Zm.9311.2.A1_x_at	3.259	CB350735	hypothetical protein LOC100274965
ZmAffx.1438.1.S1_at	3.259	11990232-4	---
Zm.3740.1.A1_at	3.238	BG840857	22.0 kDa class IV heat shock protein
Zm.2966.1.S1_at	3.216	AI001347	hypothetical protein LOC100273775
Zm.3309.1.S1_at	3.215	AW120013	---
Zm.369.1.A1_at	3.177	AF035460.1	low molecular weight heat shock protein precursor
Zm.16472.1.A1_at	3.159	AW330974	hypothetical protein LOC100273196
Zm.6380.1.A1_at	3.158	BG266095	---
Zm.6763.4.S1_at	3.156	BG841872	hypothetical protein LOC100279574
Zm.9568.1.A1_a_at	3.156	CD435747	hypothetical protein LOC100191846
ZmAffx.1306.1.S1_s_at	3.151	11990232-74	30S ribosomal protein S7
Zm.16281.1.S1_at	3.143	BM378604	---
Zm.12398.1.S1_at	3.139	BE510957	hypothetical protein LOC100275003
Zm.14419.1.A1_s_at	3.124	U76259.1	elongation factor 1-alpha
Zm.17266.1.A1_at	3.052	CF245152	---
Zm.10697.1.A1_at	3.018	AY105794.1	hypothetical protein LOC100273137
Zm.841.1.A1_at	2.977	AY110353.1	placental protein 11
Zm.18214.1.S1_at	2.963	CN845337	---
Zm.16963.1.S1_at	2.913	CF635658	voltage-gated potassium channel beta subunit
Zm.5048.6.A1_at	2.896	BM380925	endoplasmin
Zm.9140.2.A1_a_at	2.877	CF634748	---
Zm.612.1.A1_at	2.856	AF326500.1	tonoplast membrane integral protein

			ZmTIP1-2
Zm.6721.5.A1_at	2.848	CF634010	Histone H4
Zm.17198.1.A1_at	2.828	AY108769.1	BSD domain containing protein
Zm.6748.1.A1_at	2.819	BM351187	Ribosomal protein L26
Zm.6553.1.A1_at	2.811	BG265813	---
Zm.4318.1.A1_at	2.798	CA403945	hypothetical protein LOC100193401
Zm.2005.1.A1_at	2.785	BM379246	elicitor-responsive protein 1
Zm.12727.1.S1_at	2.76	CD997307	---
Zm.9249.1.A1_at	2.741	AW438186	---
Zm.2242.1.A1_at	2.724	AW330660	---
ZmAffx.949.1.A1_at	2.718	AI833412	---
Zm.11475.1.A1_a_at	2.696	BQ539206	hypothetical protein LOC100273223
Zm.10120.1.A1_at	2.695	BM380514	DNA binding like
Zm.1128.1.S1_at	2.693	BM736430	---
Zm.4868.1.S1_at	2.675	AY105012.1	SWIb domain-containing protein
ZmAffx.1333.1.S1_s_at	2.674	11990232-81	---
Zm.5050.1.S1_at	2.662	AF016305.1	ATP sulfurylase
Zm.311.1.A1_at	2.658	BM268583	18 kda heat shock protein18a
Zm.4345.1.A1_at	2.653	AW091099	hypothetical protein LOC100272516
Zm.4321.1.S1_at	2.65	AI855098	hypothetical protein LOC100191344
Zm.5322.2.A1_a_at	2.641	CF632817	hypothetical protein LOC100273032
Zm.1482.5.A1_a_at	2.632	AI714961	hypothetical protein LOC100274856
Zm.3095.1.S1_at	2.625	CO524214	hypothetical protein LOC100192080
Zm.10216.1.A1_at	2.618	CD440291	hypothetical protein LOC100276703
Zm.9547.1.A1_at	2.607	CK827410	hypothetical protein LOC100193504
Zm.12152.1.S1_at	2.604	CK394699	hypothetical protein LOC100274505
Zm.1482.1.A1_a_at	2.597	BG837937	hypothetical protein LOC100274856
Zm.12735.1.A1_at	2.588	AI491263	hypothetical protein LOC100274318
Zm.15527.1.A1_at	2.588	CK145031	hypothetical protein LOC100274385

Zm.2802.1.S1_at	2.568	CK368569	deoxyribonuclease tatD
Zm.3873.1.S1_at	2.564	AW061742	GMN10
Zm.2128.1.S1_at	2.563	AY105930.1	hypothetical protein LOC100216750
Zm.4351.1.A1_at	2.556	AI861482	hypothetical protein LOC100191604
Zm.16741.1.S1_at	2.552	AY108913.1	---
Zm.2047.1.S1_at	2.552	AJ006541	---
Zm.10423.1.S1_at	2.545	CN844467	fatty acid desaturase8
Zm.2827.1.A1_at	2.543	AY108627.1	---
Zm.490.1.S1_a_at	2.542	AF216853.1	coatomer protein2
Zm.9568.1.A1_x_at	2.512	CD435747	hypothetical protein LOC100191846
Zm.818.2.S1_at	2.509	CO531833	hypothetical protein LOC100216560
Zm.16281.1.S1_x_at	2.497	BM378604	---
Zm.14407.2.S1_at	2.483	AW424519	hypothetical protein LOC100272648
ZmAffx.1457.1.S1_at	2.482	11990232-24	---
Zm.13802.1.A1_at	2.481	AY104843.1	Zn-finger, RanBP-type, containing protein
Zm.365.1.A1_at	2.475	AB062095.1	response regulator 9
Zm.18357.1.S1_at	2.469	CF626613	hypothetical protein LOC100191155
Zm.2276.1.S1_at	2.459	CO531514	thiol protease SEN102
ZmAffx.1428.1.S1_at	2.459	11990232-13	---
Zm.19030.1.A1_at	2.457	AW179359	hypothetical protein LOC100279586
Zm.758.1.S1_at	2.457	CK827705	dehydrogenase/reductase SDR family member 2
Zm.2157.6.S1_s_at	2.455	CD439607	glycine-rich RNA-binding protein 2
Zm.13440.1.S1_at	2.454	BM379142	---
Zm.9568.1.A1_at	2.444	CD435747	hypothetical protein LOC100191846
Zm.6166.2.A1_at	2.443	CO529261	hypothetical protein LOC100278925
Zm.3507.1.A1_at	2.429	CK347589	---
Zm.6795.1.S1_at	2.411	CF632685	acid phosphatase
Zm.13424.1.A1_at	2.397	AF297044.1	homocysteine S-methyltransferase-1
Zm.4376.1.A1_at	2.394	AI712238	hypothetical protein LOC100275363

Zm.438.1.S1_at	2.394	AF133840.1	heat-shock protein 101
Zm.13595.1.A1_at	2.393	AY107750.1	phosphatase phospho1
ZmAffx.1463.1.S1_at	2.392	11990232-30	---
Zm.13316.1.S1_at	2.381	CA827638	hypothetical protein LOC100277642
Zm.1979.1.S1_at	2.38	AI948181	DNA repair protein RAD23-1
Zm.3014.1.S1_at	2.372	AY107854.1	hypothetical protein LOC100279768
Zm.17709.1.S1_at	2.371	CK369588	---
Zm.9351.2.S1_at	2.364	CF626430	transport protein particle component, Bet3 containing protein
Zm.1681.1.A1_at	2.362	CA403368	---
Zm.12227.1.S1_at	2.36	AY104350.1	heat shock cognate 70 kDa protein 2
Zm.14502.2.A1_at	2.359	BM337686	hypothetical protein LOC100279657
Zm.18208.1.A1_at	2.352	BM338077	---
Zm.2978.1.S1_at	2.352	CD981283	peptidyl-tRNA hydrolase
ZmAffx.1466.1.S1_at	2.352	11990232-33	---
Zm.5083.2.A1_a_at	2.344	AY109962.1	dnaJ protein
Zm.13630.1.S1_at	2.337	AY107387.1	hypothetical protein LOC100274007
Zm.2233.1.A1_at	2.336	CO527146	---
Zm.3551.1.S1_at	2.334	AY105376.1	---
Zm.10358.1.A1_at	2.331	CF635753	exonuclease
Zm.12423.1.S1_at	2.325	AY105422.1	reticuline oxidase
Zm.2157.6.S1_x_at	2.325	CD439607	glycine-rich RNA-binding protein 2
Zm.5283.1.A1_at	2.325	AI619100	---
Zm.16959.2.A1_at	2.318	CK347555	hypothetical protein LOC100274593
Zm.17605.1.S1_at	2.313	CK368440	---
Zm.5792.3.S1_a_at	2.309	BM499429	---
Zm.4825.1.A1_at	2.306	AI978181	hypothetical protein LOC100272679
Zm.6549.1.A1_at	2.305	BM075679	---
Zm.11595.1.A1_at	2.299	BM379199	hypothetical protein LOC100194210
ZmAffx.26.1.S1_at	2.297	AI621976	30S ribosomal protein S7

Zm.7028.1.S1_at	2.294	CF016721	hypothetical protein LOC100278953
Zm.16324.1.A1_at	2.286	CF032268	hypothetical protein LOC100194392
Zm.16504.2.A1_at	2.285	CD438012	heat shock protein18c
Zm.6069.2.S1_at	2.282	CO530423	---
ZmAffx.1489.1.S1_at	2.282	11990232-46	---
Zm.6364.1.S1_at	2.281	T20372	---
Zm.3498.1.A1_a_at	2.277	CK367906	metallothionein1
Zm.12426.1.S1_at	2.276	AY111182.1	---
Zm.782.1.A1_at	2.267	CA404412	hypothetical protein LOC100273031
ZmAffx.1513.1.S1_at	2.266	11990232-53	---
Zm.5785.4.A1_at	2.256	CA403256	hypothetical protein LOC100279822
Zm.4679.1.S1_at	2.251	AI737934	pre-rRNA-processing protein TSR2
ZmAffx.1143.1.A1_at	2.249	AW399931	---
Zm.9549.2.A1_a_at	2.246	BM332597	hypothetical protein LOC100191610
Zm.611.1.A1_at	2.242	AF326499.1	small basic membrane intrinsic protein2a
Zm.16498.3.A1_x_at	2.24	CK368372	hypothetical protein LOC100193260
Zm.17806.1.A1_at	2.239	CF634845	---
Zm.12760.1.A1_at	2.237	CF637010	catalytic/ hydrolase
Zm.10337.1.S1_at	2.236	CO522438	L-lactate dehydrogenase A
Zm.2176.1.S1_at	2.236	AY111425.1	---
Zm.17669.1.S1_at	2.231	CF636811	---
Zm.7750.2.S1_at	2.23	AY103542.1	Beta-6 tubulin
Zm.17778.1.S1_at	2.229	CK370487	---
Zm.6666.2.S1_at	2.227	BI478864	26S proteasome non-ATPase regulatory subunit 8
Zm.5124.1.S1_at	2.222	CA405206	ubiquitin-protein ligase
ZmAffx.581.1.A1_at	2.218	AI714922	---
Zm.13816.1.A1_at	2.216	AY104641.1	hypothetical protein LOC100276882
Zm.17712.1.S1_at	2.215	CK370052	hypothetical protein LOC100275406
Zm.3998.1.S1_at	2.208	AY108495.1	hypothetical protein LOC100278863

Zm.19106.1.A1_at	2.203	CO049276	---
Zm.15576.1.A1_x_at	2.197	CK827910	---
Zm.3791.1.A1_at	2.197	AW224984	---
Zm.13241.1.S1_at	2.196	AY059647.1	alternative oxidase AOX2 precursor
Zm.7540.1.S1_at	2.186	CF055286	hypothetical protein LOC100192075
Zm.537.1.A1_a_at	2.185	AI065500	hypothetical protein LOC100194026/LOC100273405
Zm.12549.1.A1_at	2.182	AI649492	mitochondrial aspartate-glutamate transporter AGC1
Zm.15874.1.A1_at	2.172	BM340119	---
Zm.18594.1.A1_at	2.17	CO519739	prMC3
Zm.13766.1.S1_at	2.167	CF630318	annexin A4
Zm.17339.2.A1_a_at	2.16	CF633149	---
Zm.14932.1.A1_at	2.154	BI993187	---
Zm.6404.1.A1_at	2.154	AY110082.1	---
Zm.15514.1.A1_at	2.152	CD437159	hypothetical protein LOC100216604
Zm.995.1.A1_at	2.147	CF040767	---
Zm.14302.2.A1_a_at	2.144	AY109645.1	proteasome subunit alpha type 3
Zm.12387.1.S1_at	2.143	AW000286	---
Zm.1691.2.S1_a_at	2.143	AW681248	hypothetical protein LOC100191436
Zm.7750.2.S1_x_at	2.14	AY103542.1	Beta-6 tubulin
Zm.13332.1.S1_at	2.139	AY105769.1	ribosomal protein L32 containing protein
Zm.5391.2.A1_a_at	2.139	BM336235	2-cys peroxiredoxin BAS1
Zm.5833.1.A1_at	2.135	CA398697	adenylate kinase
Zm.9169.2.S1_at	2.133	CO521754	hypothetical protein LOC100194136
Zm.12547.1.A1_at	2.132	AI622168	RNA binding protein
Zm.19137.1.A1_at	2.126	CO523276	---
Zm.3851.1.A1_at	2.122	CK394813	---
Zm.14513.1.A1_at	2.116	CA828306	Histone H3.2
Zm.13217.1.A1_at	2.114	AI065901	hypothetical protein LOC100273064

Zm.12420.1.S1_at	2.113	BF728806	---
Zm.1934.1.S1_at	2.112	AW519956	glycerophosphodiester phosphodiesterase
Zm.14316.1.S1_at	2.108	AY104671.1	hypothetical protein LOC100274358/LOC100277073
Zm.2447.1.A1_at	2.108	BM079996	---
ZmAffx.963.1.A1_at	2.107	AI833792	---
Zm.6532.1.A1_at	2.104	U09276.1	abscisic stress protein homolog
ZmAffx.675.1.A1_at	2.097	AI734494	---
Zm.4225.1.S1_at	2.087	AW225317	hypothetical protein LOC100192639
Zm.13809.1.A1_at	2.08	AY104739.1	hypothetical protein LOC100275407
Zm.4431.1.S1_at	2.076	AI714808	protein phosphatase methylesterase 1
Zm.16498.3.A1_at	2.074	CK368372	Hypothetical protein LOC100193260
Zm.3855.1.S1_at	2.074	AA979788	---
Zm.9595.1.A1_at	2.073	BM333398	seven transmembrane domain protein
Zm.12376.1.S1_at	2.065	BQ577893	---
Zm.5141.2.A1_a_at	2.063	AI691820	hypothetical protein LOC100191902/LOC100273101
ZmAffx.1224.1.S1_at	2.063	11990232-115	---
Zm.5486.1.A1_at	2.062	AI629867	---
Zm.13988.1.S1_at	2.061	AY103582.1	hypothetical protein LOC100279316
Zm.12216.1.S1_at	2.053	CF050683	---
ZmAffx.1498.1.S1_s_at	2.052	40794996-115	---
Zm.3838.1.A1_at	2.05	CF063291	---
Zm.6748.1.A1_x_at	2.05	BM351187	Ribosomal protein L26
Zm.8247.1.A1_at	2.049	BM073399	---
ZmAffx.1453.1.S1_at	2.049	11990232-20	---
Zm.19099.1.S1_at	2.047	CO530975	---
Zm.2323.1.A1_at	2.044	CO526297	hypothetical protein LOC100273265

Zm.6397.1.A1_at	2.043	AI691676	---
Zm.505.2.S1_a_at	2.04	AI665681	glucose translocator1
Zm.3098.1.A1_at	2.039	BM379893	caffeoyl-CoA O-methyltransferase 1
Zm.3719.1.A1_at	2.039	CK370903	---
Zm.12534.1.A1_at	2.038	CF628471	hypothetical protein LOC100192944
Zm.5181.1.A1_at	2.033	CF030446	CK2 protein kinase alpha 2
Zm.3476.1.A1_at	2.032	BQ538179	---
Zm.2000.1.A1_at	2.031	AY110879.1	---
Zm.6155.1.A1_a_at	2.029	T20358	hypothetical protein LOC100273594
Zm.14302.2.A1_x_at	2.02	AY109645.1	proteasome subunit alpha type 3
Zm.6130.1.S1_at	2.02	CF056233	Hypothetical protein LOC100216775
Zm.5496.1.S1_at	2.016	AY105645.1	hypothetical protein LOC100275844
Zm.18525.1.A1_at	2.015	CO530497	---
Zm.4517.1.A1_at	2.015	AY105906.1	hypothetical protein LOC100191201
Zm.8107.1.A1_at	2.013	CK827080	---
Zm.9952.1.A1_at	2.004	BM349898	---
Zm.3219.3.S1_at	2.003	CF056029	thiamine biosynthesis protein thiC
Zm.14532.1.A1_a_at	2.001	AY172634.2	isoamylase-type starch debranching enzyme ISO3
Zm.6207.6.S1_at	2.001	BM895377	60S ribosomal protein L15
Zm.8581.1.A1_at	0.5	BM267381	hypothetical protein LOC100216584
Zm.9696.1.S1_at	0.5	BM337562	---
Zm.5853.1.A1_at	0.499	AI649903	hypothetical protein LOC100193409
Zm.16259.1.A1_x_at	0.498	BQ539455	hypothetical protein LOC100273269
Zm.7012.8.A1_x_at	0.498	BU092758	ribosomal protein S8
Zm.16232.1.A1_a_at	0.497	BG842260	hypothetical protein LOC100278121
Zm.18066.1.A1_s_at	0.496	AI738164	hypothetical protein LOC100272811
Zm.1322.1.A1_at	0.495	AI737263	hypothetical protein LOC100279212
Zm.15480.1.S1_at	0.495	BM349789	---

Zm.16142.1.S1_at	0.495	CF243882	hypothetical protein LOC100194402
Zm.2483.1.A1_at	0.492	BG841687	hypothetical protein LOC100275206
Zm.6569.2.S1_a_at	0.491	AY107413.1	hypothetical protein LOC100276303
Zm.7025.1.A1_at	0.488	CF047868	hypothetical protein LOC100274924
Zm.858.1.A1_at	0.488	BM073310	hypothetical protein LOC100216691
Zm.16508.3.A1_x_at	0.487	AI739900	ribosomal protein L39
Zm.14398.2.A1_a_at	0.486	CK985944	protein OS-9/protein OS-9
Zm.16228.1.S1_a_at	0.486	BU197473	hypothetical protein LOC100275186
Zm.14833.1.A1_at	0.485	CF629020	---
ZmAffx.1217.1.S1_s_at	0.484	40794996-164	---
Zm.466.1.S1_at	0.483	AI622469	Acidic ribosomal protein P2a-4
Zm.10850.1.S1_at	0.482	AY106263.1	ZIM motif family protein
Zm.16936.1.A1_at	0.482	AY108717.1	---
Zm.14531.1.S1_at	0.481	AY111968.1	invertase cell wall3
Zm.2412.1.S1_at	0.481	BM349568	hypothetical protein LOC100192774
Zm.3475.1.S1_at	0.481	CF002596	ribosomal protein S27
Zm.4553.2.S1_a_at	0.481	AI734766	---
Zm.5661.1.A1_at	0.481	AY107527.1	---
Zm.14934.1.A1_at	0.479	CK986163	CHCH domain containing protein
Zm.4212.2.S1_a_at	0.478	BI543125	60S ribosomal protein L22-2
Zm.4949.1.A1_at	0.478	BM379432	---
Zm.733.1.S1_at	0.478	AY108731.1	SINA2
Zm.312.1.A1_at	0.477	S72425.1	ADP-glucose pyrophosphorylase
Zm.13787.1.S1_at	0.476	AY105138.1	---
ZmAffx.1298.1.S1_at	0.476	40794996-139	---
ZmAffx.1535.1.S1_at	0.476	40794996-15	---
Zm.10097.1.A1_at	0.472	BQ538355	cupin, RmlC-type

Zm.9791.1.S1_at	0.471	AY110405.1	hypothetical protein LOC100216896
ZmAffx.1405.1.S1_at	0.471	40794996-80	---
Zm.3743.2.A1_a_at	0.47	CF624095	hypothetical protein LOC100274177
Zm.4784.1.A1_at	0.468	BM338307	hypothetical protein LOC100277212
Zm.12022.1.A1_at	0.467	BM382727	---
Zm.19125.1.S1_at	0.466	CF631194	hypothetical protein LOC100278829
ZmAffx.1358.1.S1_at	0.466	40794996-159	---
Zm.13307.1.S1_at	0.465	CA827415	---
Zm.2208.1.S1_at	0.465	CO527853	malonyl CoA-acyl carrier protein transacylase
Zm.3183.1.A1_at	0.464	CF634309	hypothetical protein LOC100274988
Zm.11428.1.A1_at	0.463	CF244302	hypothetical protein LOC100274452
Zm.6650.1.A1_at	0.463	BM336595	fiber protein Fb2
Zm.1250.2.S1_at	0.462	AY104390.1	Hypothetical protein LOC100191515
ZmAffx.1416.1.S1_at	0.462	40794996-91	---
Zm.10324.1.A1_at	0.461	BQ048175	---
Zm.15432.1.S1_at	0.459	CA401222	---
Zm.17260.1.A1_at	0.459	CK145375	hypothetical protein LOC100277879
Zm.13578.1.A1_at	0.458	AY107890.1	---
Zm.1554.1.S1_a_at	0.458	AW287806	hypothetical protein LOC100275340/LOC100276203
Zm.8540.1.A1_at	0.458	BM079841	hypothetical protein LOC100193457
Zm.5417.1.A1_at	0.455	CA403570	hypothetical protein LOC100193358
Zm.15811.1.S1_at	0.454	CF633035	RNA binding protein
Zm.9980.1.A1_at	0.452	BM331957	---
Zm.17253.1.S1_at	0.451	CA398537	---
Zm.4543.1.S1_at	0.451	CF628165	hypothetical protein LOC100192734
Zm.13304.1.S1_at	0.45	CA827334	---
Zm.1807.1.A1_at	0.448	AW453229	hypothetical protein LOC100279285

Zm.280.1.A1_at	0.446	U58208.1	Binding protein homolog1
Zm.15288.1.A1_at	0.445	CK369497	hypothetical protein LOC100276056
Zm.650.1.S1_at	0.445	AY029556.1	methyl-binding domain protein MBD101
Zm.2812.1.S1_at	0.444	AW067476	---
ZmAffx.1479.1.S1_at	0.443	40794996-106	---
Zm.10091.1.A1_at	0.442	BM382104	hypothetical protein LOC100277849
Zm.15320.2.S1_x_at	0.439	CF633376	---
Zm.4836.1.A1_at	0.437	BM078446	fb14
Zm.3900.1.S1_at	0.435	BM073698	---
Zm.4361.1.A1_at	0.434	CD436511	hypothetical protein LOC100192530
Zm.16259.3.A1_x_at	0.433	AI395995	hypothetical protein LOC100273269
Zm.17594.1.S1_at	0.433	CA402152	hypothetical protein LOC100276455
ZmAffx.790.1.A1_at	0.433	AI759115	---
Zm.7976.1.S1_at	0.432	BM080696	hypothetical protein LOC100217261
ZmAffx.1326.1.S1_at	0.432	40794996-148	---
Zm.16524.1.S1_at	0.431	AW054294	hypothetical protein LOC100193178
Zm.3412.1.S1_at	0.431	CA405066	---
Zm.13715.1.A1_at	0.429	AY106496.1	hypothetical protein LOC100276156
Zm.2441.1.S1_s_at	0.428	BE510252	CFM6
Zm.7095.1.A1_a_at	0.428	CK371352	hypothetical protein LOC100194044
Zm.2997.1.S1_at	0.426	CD983066	REX1 DNA Repair family protein
Zm.19203.1.S1_at	0.425	CO520849	hypothetical protein LOC100193957
Zm.5549.1.A1_at	0.423	CK144503	hypothetical protein LOC100273359
ZmAffx.831.1.S1_at	0.423	AI770541	hypothetical protein LOC100191667
ZmAffx.548.1.A1_at	0.421	AI712133	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase
Zm.4615.2.A1_at	0.418	AI714747	---
Zm.14154.1.A1_s_at	0.411	BM073783	gibberellin-regulated protein 2

Zm.206.4.A1_s_at	0.408	CF048188	ribosomal protein S21/ 40S ribosomal subunit protein S21
Zm.7012.7.A1_a_at	0.408	AI881466	ribosomal protein S8
Zm.8492.1.A1_at	0.408	CO518733	cyclin-dependent kinases regulatory subunit
Zm.9905.1.A1_at	0.403	AY107944.1	IDP680 protein/ RAB5-interacting protein
Zm.1701.1.S1_at	0.401	AW399969	hypothetical protein LOC100280325
Zm.1784.1.S1_at	0.397	BF728627	hypothetical protein LOC100277524
Zm.14013.1.A1_a_at	0.392	BM075523	hypothetical protein LOC100193147/LOC100275870/ LOC100276632/ LOC100278726
Zm.15004.1.S1_at	0.392	BG840536	caltractin
Zm.2165.1.A1_at	0.392	BM072875	hypothetical protein LOC100278701
ZmAffx.1355.1.S1_at	0.39	40794996-45	---
Zm.10500.1.S1_at	0.388	CA827201	---
Zm.18336.1.A1_at	0.388	CO520030	---
Zm.678.1.S1_at	0.387	AF302187.1	sucrose export defective1
Zm.6662.1.A1_a_at	0.386	AY108391.1	50S ribosomal protein L20
Zm.13941.1.S1_at	0.385	CB380998	hypothetical protein LOC100277314
Zm.18141.1.S1_at	0.381	CF024422	---
Zm.6298.1.A1_at	0.375	CD444675	hypothetical protein LOC100278812
Zm.18062.1.A1_at	0.371	CO526041	hypothetical protein LOC100192968
Zm.10299.1.S1_at	0.369	AW172106	---
Zm.3284.1.A1_at	0.369	BM952816	---
Zm.16475.2.S1_at	0.367	CK371021	---
Zm.17336.1.A1_at	0.366	CF632816	---
Zm.19304.1.A1_at	0.366	CO525321	hypothetical protein LOC100192965
Zm.4326.1.A1_at	0.366	AI711615	hypothetical protein AY106635
Zm.8142.1.A1_at	0.366	BM080424	hypothetical protein LOC100275043
ZmAffx.1294.1.S1_at	0.365	40794996-137	---

Zm.7569.1.A1_at	0.362	BM347275	---
Zm.10294.1.S1_at	0.361	CD944058	---
Zm.9710.1.S1_at	0.361	CF624968	hypothetical protein LOC100276125
Zm.8233.3.S1_a_at	0.359	BQ294345	splicing factor, arginine/serine-rich 7
Zm.8925.1.A1_at	0.359	BM381305	---
Zm.13825.1.S1_at	0.353	AY104501.1	---
Zm.3781.1.S1_at	0.348	CD447284	syntaxin 132
Zm.12654.1.S1_at	0.347	BU098714	---
Zm.13600.1.A1_at	0.346	CD440740	surfeit locus protein 5
Zm.4312.2.S1_at	0.344	AY107006.1	---
Zm.12030.1.A1_at	0.343	CF623978	---
Zm.16501.1.S1_at	0.335	AY108566.1	---
Zm.14412.1.S1_a_at	0.331	BM337383	hypothetical protein LOC100194289
Zm.9733.2.A1_a_at	0.331	BM337265	nuclear transcription factor Y subunit A-1
Zm.3248.1.A1_at	0.33	BG842948	hypothetical protein LOC100278584
Zm.14376.1.S1_at	0.326	BG836683	diphosphomevalonate decarboxylase
Zm.1291.1.S1_at	0.325	CO533162	hypothetical protein LOC100194121
ZmAffx.49.1.A1_at	0.325	AI664982	---
Zm.2514.1.A1_at	0.324	CO524312	hypothetical protein LOC100273294
ZmAffx.1219.1.S1_s_at	0.323	40794996-166	---
Zm.18685.1.A1_at	0.318	CO532513	hypothetical protein LOC100194065
Zm.4553.1.S1_at	0.318	BE238641	---
Zm.6023.1.S1_at	0.313	AY059648.1	alternative oxidase AOX3 precursor
Zm.585.1.S1_at	0.31	AF180131.1	female gametophyte-specific protein ES1 precursor
Zm.6838.1.A1_at	0.31	CF035294	---
Zm.5766.1.S1_x_at	0.305	BG840404	---
Zm.2884.1.S1_at	0.303	AW090890	hypothetical protein LOC100192085

Zm.6208.1.A1_at	0.303	CO527309	hypothetical protein LOC100273615
Zm.1451.2.S1_x_at	0.302	CF021148	calmodulin
Zm.8921.1.A1_at	0.3	BM075327	hypothetical protein LOC100217070
Zm.4312.2.S1_at	0.3	AY107006.1	---
Zm.13601.1.S1_at	0.297	AY107674.1	---
Zm.17267.1.S1_at	0.297	CK368940	hypothetical protein LOC100275082
Zm.3135.1.S1_at	0.297	CD981826	catalytic/ protein phosphatase type 2C
Zm.1747.1.A1_at	0.295	CF633743	---
Zm.889.2.S1_at	0.294	CD439290	---
Zm.14441.1.A1_at	0.291	CO531121	HAD-superfamily hydrolase, subfamily IIA
Zm.16590.3.A1_at	0.289	AY104237.1	DNA-directed RNA polymerases I, II, and III 14.4 kDa polypeptide
ZmAffx.1376.1.S1_s_at	0.284	40794996-51	---
Zm.6605.1.A1_at	0.283	AI444684	hypothetical protein LOC100191572
ZmAffx.1352.1.S1_at	0.279	40794996-156	---
Zm.17931.1.A1_at	0.271	CK827676	hydrogen-transporting ATP synthase, rotational mechanism
Zm.11918.1.A1_at	0.27	BM381808	hypothetical protein LOC100274802
Zm.11646.1.A1_at	0.269	CO523950	hypothetical protein LOC100273629
Zm.16904.2.A1_a_at	0.269	BM381185	---
Zm.10452.1.A1_at	0.268	CK826753	hypothetical protein LOC100275165
Zm.11748.1.A1_at	0.266	BM380426	hypothetical protein LOC100276902
Zm.17274.1.A1_at	0.263	CK371488	---
Zm.18350.1.A1_at	0.255	CO524599	---
ZmAffx.1474.1.S1_s_at	0.252	40794996-101	---
Zm.12528.1.A1_at	0.251	AI734581	hypothetical protein LOC100274560

Zm.955.1.A1_at	0.25	BM339428	e2 protein
Zm.12980.1.A1_at	0.241	CA402690	---
Zm.4069.1.A1_at	0.239	CO532116	hypothetical protein LOC100274464
Zm.17145.1.S1_at	0.235	AY211982.1	transparent leaf area peptide
Zm.5823.1.A1_at	0.228	CO520297	hypothetical protein LOC100194104
Zm.17821.1.S1_at	0.218	CD961542	myo-inositol kinase
Zm.3668.1.A1_at	0.218	AI649862	---
Zm.4611.1.A1_at	0.218	BG842817	IDP139 protein
Zm.11638.1.S1_at	0.211	BM379507	---
Zm.13299.1.S1_at	0.211	BQ486451	---
Zm.1451.2.S1_at	0.209	CF021148	calmodulin
Zm.6598.1.A1_at	0.206	AI737222	gibberellin-regulated protein 2
Zm.4118.1.A1_at	0.204	AY105314.1	IDP1681 protein
Zm.2040.1.S1_at	0.189	BM338990	hypothetical protein LOC100194284
Zm.4083.1.A1_at	0.188	CA403575	hypothetical protein LOC100191657
Zm.12422.1.S1_at	0.181	AW400260	---
Zm.10247.1.S1_at	0.178	BU049731	---
Zm.18590.1.A1_at	0.176	AW562614	exostosin-like
Zm.3174.1.A1_at	0.176	BM072921	AP-2 complex subunit sigma-1
ZmAffx.466.1.S1_at	0.175	AI677405	---
Zm.12622.1.A1_at	0.171	BU051177	hypothetical protein LOC100278739
Zm.2695.1.S1_at	0.164	CN844861	hypothetical protein LOC100279628
Zm.2397.1.A1_at	0.16	AY104240.1	nucleolin
Zm.9832.1.A1_at	0.12	BM340381	hypothetical protein LOC100279963
Zm.2345.1.S1_at	0.117	BF727904	---
Zm.4056.1.A1_at	0.104	CA403994	hypothetical protein LOC100192464
Zm.15759.1.A1_at	0.097	AW129802	hypothetical protein LOC100277415
Zm.14265.1.A1_at	0.081	AY105423.1	hypothetical protein LOC100272919
Zm.7750.4.S1_at	0.076	AW499152	---

Zm.15602.1.S1_at	0.06	CA399213	---
Zm.16232.1.A1_x_at	0.059	BG842260	hypothetical protein LOC100278121
ZmAffx.92.1.A1_at	0.043	AI665387	---
Zm.16259.3.A1_a_at	0.027	AI395995	hypothetical protein LOC100273269
Zm.10455.1.S1_at	0.023	BQ577982	---
Zm.11825.1.S1_at	0.018	BM381071	hypothetical protein LOC100276984
ZmAffx.466.1.A1_at	0.018	AI677405	---
Zm.17801.1.S1_at	0.015	CK371451	---

“---” represents the genes have no annotations.

Supplemental Table S3: List of primers used for Microarray analysis

Primer name	Primer sequence
AI714808f	5’- ATGACGAGCTGATCCTGCTT-3’
AI714808r	5’- ATGTCGCATGAGTTGCTTTG-3’
CD438012f	5’-AGGGAGCAAGCAGTAGCTGT-3’
CD438012r	5’-TGGCGACACGATCTTCACTA-3’
AY172634	5’-GCTAATCGTTTTGCGGAGAC-3’
AY172634	5’-CCCATTTCCTTGGTCACACT-3’
AF302187f	5’- CTCCTTTACCTCCCCCTCAG-3’
AF302187r	5’-GCGTGGGCGATATAACAACT-3’
CF021148f	5’-AGTTGCTGAAAATGGCAATG-3’
CF021148r	5’-TGTGGGTGGGGTGAAACTA-3’