

Note

Transcriptomic profiles of nodule senescence in *Lotus japonicus* and *Mesorhizobium loti* symbiosis

Sirinapa Chungopast^{1,2}, Hideki Hirakawa³, Shusei Sato⁴, Yoshihiro Handa⁵,
Katsuhiro Saito⁶, Masayoshi Kawaguchi⁵, Shigeyuki Tajima¹, Mika Nomura^{1,*}

¹Faculty of Agriculture, Kagawa University, Kita-gun, Kagawa 761-0795, Japan; ²Faculty of Agriculture, Kasetsart University, Nakorn Pathom 73140, Thailand; ³Kazusa DNA Research Institute, Kisarazu, Chiba 292-0818, Japan; ⁴Tohoku University, Sendai, Miyagi 980-8577, Japan; ⁵Division of Symbiotic Systems, National Institute for Basic Biology, Okazaki, Aichi 444-8585, Japan; ⁶Faculty of Agriculture, Shinshu University, Kamiina-gun, Nagano 399-4598, Japan

* E-mail: nomura@ag.kagawa-u.ac.jp Tel: +81-87-891-3135 Fax: +81-87-891-3021

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Abstract Nodule senescence is a complex developmental process during which essential nutrients are recycled. In order to understand the regulatory mechanism, transcript-profiling analysis during nodule senescence was performed in the *Lotus japonicus*-*Mesorhizobium loti* symbiosis. Microarray data showed significantly up-regulated expressions in 641 genes out of a total of 20,165 genes during nodule senescence, and down-regulated expressions were observed in 416 genes. These up-regulated genes during senescence were related to cell wall/membrane/envelope biogenesis and extracellular structures. Down-regulated genes were mainly responsible for defense mechanisms. We classified senescence up-regulated genes in two clusters. Genes in cluster 1 were induced at senescence specific stage and those in cluster 2 were induced from nitrogen fixation stage and expressed until nodule senescence. The genes in cluster 1 included typical marker for senescence like gene for heat shock protein. Four hundred sixteen down-regulated genes during nodule senescence were also classified in two clusters, cluster 3 and cluster 4. These genes corresponded to metabolisms for amino acid and plant hormones which are necessary for growth and cell division during nodule development and nitrogen fixation. These results provide the comprehensive data source for investigation of molecular mechanisms underlying nodule senescence in *Lotus japonicus*-*Mesorhizobium loti* symbiosis.

Key words: *Lotus japonicus*, *Mesorhizobium loti*, nodule senescence, symbiosis, transcriptome.

The symbiotic nitrogen-fixing nodules are established and decayed by complex interactions between legume plants and Rhizobia. The nitrogen fixing activity of the nodule increased from 12 days post inoculation in *Lotus japonicus* (Kouchi et al. 2004) and the nitrogen fixing capacity of the nodules peaked early in its life when the nodule is 3 to 5 weeks post inoculation (wpi, Puppo et al. 2005). Such nodule senescence has been reported to be triggered by various types of stress. The activity of enzymes used in the hydrolysis process associating with programmed cell death increased, especially cysteine proteases and H₂O₂ generating enzymes (Alesandrini et al. 2003; Muller et al. 2001). During nodule senescence, pink color of nodules turns to green because hemes of leghemoglobin are nitrated (Navascués et al. 2012). However, the regulatory mechanisms that govern nodule senescence still remain elusive.

Nodules can be developmentally classified into two main groups. Indeterminate nodules, as observed in *Medicago truncatula*, form cylindrical nodules that

consist of a gradient of developmental zones with a persistent apical meristem; an infection zone, a fixation zone, and a senescence zone. Aged nodules with those three zones of *M. truncatula* were monitored by microarray (Van de Velde et al. 2006). In the case of *Lotus japonicus*, determinate nodules which are round organs with a central fixation zone were formed. A large-scale gene expression profile was reported using a cDNA array during the early stages of the *L. japonicus* nodulation process (Colebatch et al. 2002; Kouchi et al. 2004). They described the nodule-induced genes, which are involved in the symbiotic nitrogen fixation. However, there is no microarray report of senescence nodule in *L. japonicus*.

In this paper, we present a detailed analysis of nodule senescence in *L. japonicus*.

L. japonicus plants inoculated with *M. loti* were grown in a growth cabinet (EYELA FLI-2000) at 24°C and exposed to 16-h/light and 8-h/dark cycles. Total RNA was isolated from nodules at 2, 4 and 8 wpi using an RNeasy Plant mini kit (Qiagen), respectively.

The acetylene reduction activity of the nodules was surveyed at 2, 4, 8 wpi in *L. japonicus*, and values were 12.28 ± 3.27 , 54.83 ± 13.17 , 15.04 ± 2.60 nmol h⁻¹ plant⁻¹, respectively. The nodules at 2 wpi, 4 wpi and 8 wpi represent early nitrogen fixation stage, mature nitrogen fixation stage and senescence stage, respectively. Extracted total RNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop) and checked for quality using an Agilent 2100 Bioanalyzer (Agilent Technologies (CA, USA)). Labeling was performed using a Quick Amp labeling kit, One-color Cy3-CTP (Agilent Technologies) according to the manufacturer's protocol.

The Agilent 4×44 microarray chip was designed for *Lotus* nodule transcript sequences. We selected 20,165 genes which were expressed in nodulated roots at 12 days post inoculation by RNA-seq analysis (Handa et al. unpublished data). Two probes per target gene were designed by the eArray (Agilent Technologies). Each probe was a 60-mer oligonucleotide and the 4×44 K format Agilent microarray consisted of 40,330 probes. The Agilent microarray probes and gene ID are available from GEO (Platform Approval, GPL18811).

For microarray hybridization, 1,650 ng of Cy3-labeled cRNAs of nodules at 2, 4 and 8 wpi were fragmented and hybridized on a slide of *Lotus* 4×44 K microarray at 65°C for 17h. Hybridization and washing of the hybridized slides were performed according to the manufacturer's instructions. Slides were scanned on the Agilent microarray scanner. The processed raw signal intensity of all probes (40,330) was subjected to 75 percentile normalization with GeneSpring for inter-array comparison (Agilent Technologies) and transformed to log 2 scale.

To isolate genes that are differentially expressed during nodule senescence, the transcriptome data of young nodules at 2 wpi was compared with that of senescence nodule at 8 wpi. The significant intensity differences of more than two-fold and less than 1/2 fold compared to nodules at 2 wpi were listed as up-regulated and down-regulated genes, respectively. The microarray data showed that 641 out of a total of 20,165 genes were up-regulated genes and 416 genes were down-regulated. To investigate the functional distribution, the identified genes were classified into 25 major functional categories according to the euKaryotic Orthologous Groups (KOGs) of NCBI (Figure 1). Among a total of 641 up-regulated genes, 55.4% of the genes displayed similarity to the sequences with known or putative functions. We classified total 20165 genes by KOG database of NCBI. Based on the classification, Figure 1 shows the percentage of each classified up- or down-regulated genes. Significantly up-regulated genes during senescence were related to cell wall/membrane/envelope biogenesis (4.45%) or extracellular structures (6.02%) were identified. These up-regulated genes were

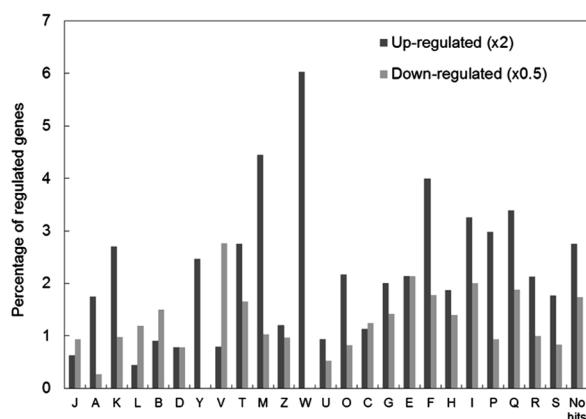
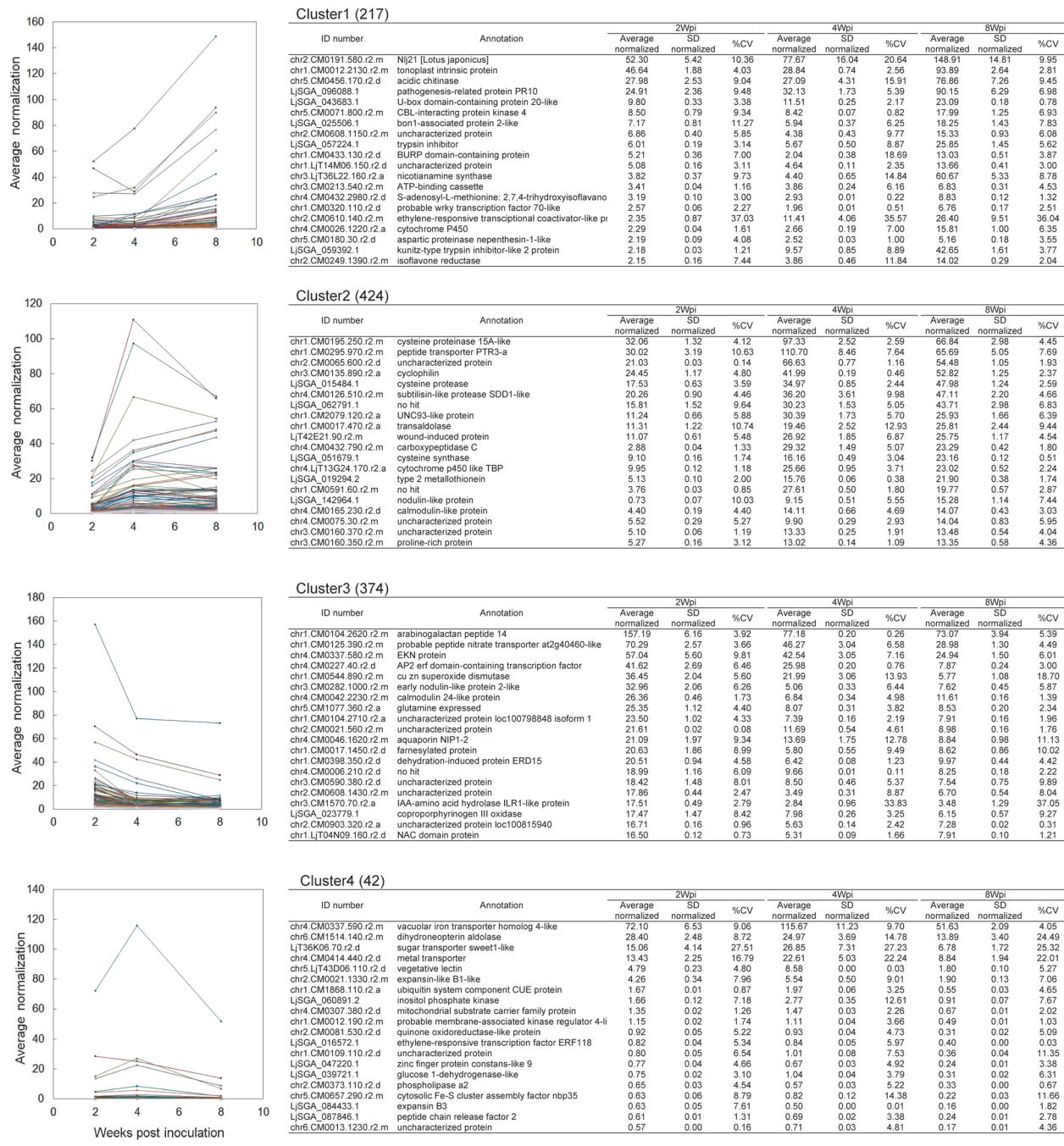


Figure 1. Functional classification according to euKaryotic Orthologous Groups (KOGs) database. Relative frequency of genes belonging to a category is given for 2 data sets: The expression of up-regulated genes (■) and down-regulated genes (■) were more than two-fold and less than 1/2-fold nodules at 8 wpi compared to nodules at 2 wpi, respectively. J: translation, ribosomal structure and biogenesis, A: RNA processing and modification, K: transcription, L: replication, recombination and repair, B: chromatin structure and dynamics, D: cell cycle control, cell division, chromosome partitioning, Y: nuclear structure, V: defense mechanisms, T: signal transduction mechanisms, M: cell wall/membrane/envelope biogenesis, Z: cytoskeleton, W: extracellular structures, U: intracellular trafficking, secretion, and vesicular transport, O: posttranslational modification, protein turnover, chaperones. C: energy production and conversion, G: carbohydrate transport and metabolism, E: amino acid transport and metabolism, F: nucleotide transport and metabolism, H: coenzyme transport and metabolism, I: lipid transport and metabolism, P: inorganic ion transport and metabolism, Q: secondary metabolites biosynthesis, transport and catabolism, R: general function prediction only, S: function unknown, No hits. The data represents percentage of number of up- or down regulated genes in each KOGs per number of genes in each classified KOGs.

divided into two clusters, cluster 1 and cluster 2 (Figure 2, Supplemental Tables 1 and 2). Their expression profile of cluster 1 was significantly induced at 8 wpi. The genes of cluster 2 were induced from 4 wpi and continuously expressed until 8 wpi.

Genes classified in cluster 1 and cluster 2 were 217 and 424, respectively (Supplemental Tables 1 and 2). Cluster 1 had noticeable genes for the Kunitz-type trypsin inhibitor (LjSGA_059392.1) and NAC domain-containing proteins (chr3.CM0460.20.r2.d, Figure 2, Supplemental Table 1). The protease inhibitor of the Kunitz family is important in strictly controlling the synthesis of protease (Habib and Fazili 2007). NAC domain-containing protein, GmNAC6, is synergistically activated by a combination of endoplasmic reticulum (ER) stress and osmotic stress signals and induces a senescence-like response and cell death in soybean (Faria et al. 2011). The NAC domain-containing protein in cluster 1 of *L. japonicus* may also induce rapid cell death. The genes in cluster 1 also contained a typical marker for the senescence like a heat shock protein (chr4.CM0432.1970.r2.m).

The serine acetyltransferase gene (chr6.CM0245.580.



r2.a), which catalyzes acetyl-CoA to O-acetylserine, was classified in cluster 2. Genes for metallothionein (LjSGA_019294.2) and heat shock protein (chr3.CM0127.770.r2.d) were also in cluster 2. These genes were induced under oxidative stress or defense response (Büttner and Singh 1997; Cvjetko et al. 2014; Hondo et al. 2007; Mehta et al. 1996; Mir et al. 2004). A gene for iron-storage protein, ferritin (chr3.CM0116.300.r2.m), was classified in cluster 2 (Supplemental Table 2, Figure 3). During nodule senescence, membranes in nodule tissue degrade and iron is released to cytoplasm, inhibiting nitrogen fixation (Arosio et al. 2009; Ravet et al. 2009). Increased ferritin observed within the nodules might reduce iron content in cytoplasm and restrict induced senescence, resulting in prolonged nitrogen fixation. These genes classified in cluster 2 might be

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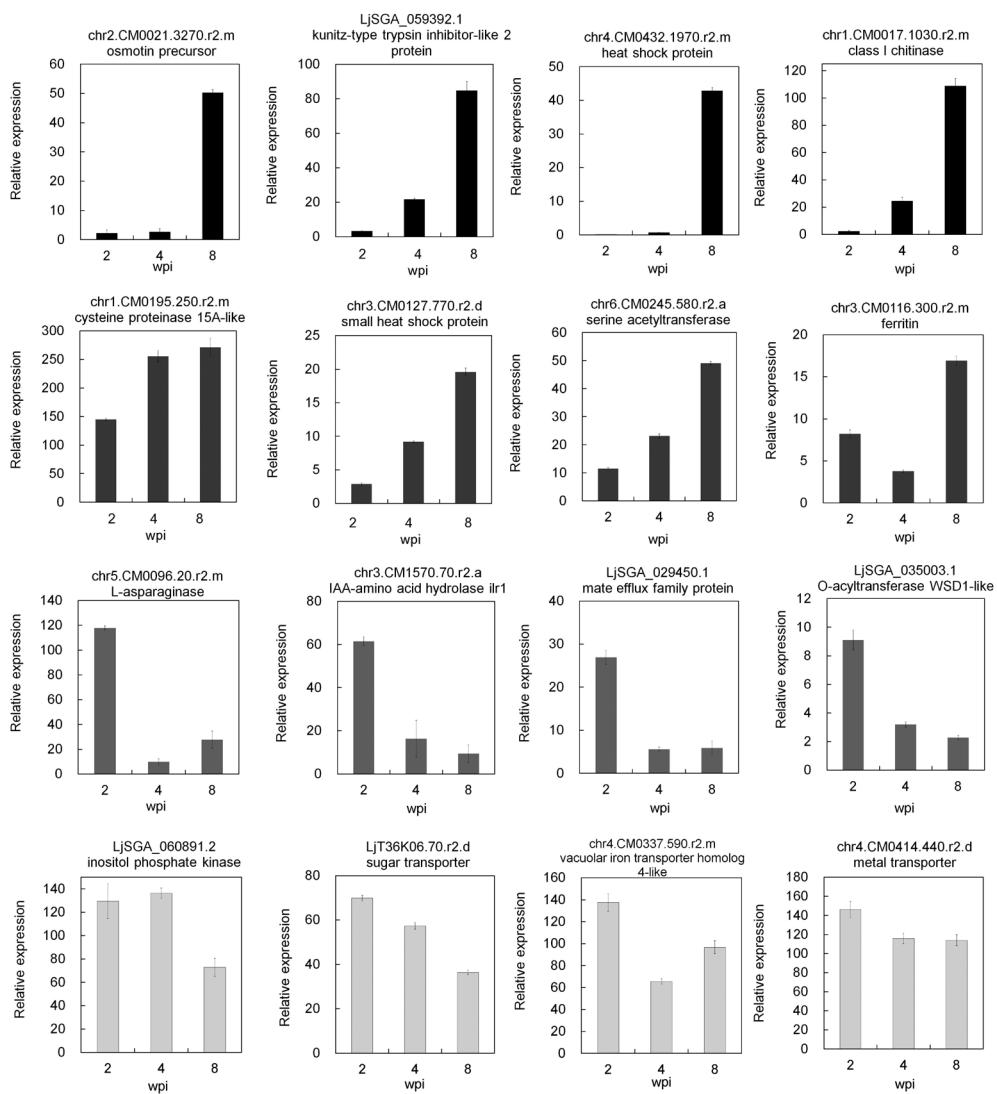


Figure 3. Comparison of relative expression levels in the microarray analysis with qRT-PCR. Representatives of genes in cluster 1 (■), cluster 2 (■), cluster 3 (■) and cluster 4 (■) during nodule development. The expression profile was normalized to ubiquitin. All of the data are shown as \pm SD wpi: weeks post inoculation.

important to maintain nitrogen fixation in the nodules.

Four hundred sixteen down-regulated genes during nodule senescence were also classified into two clusters, cluster 3 and cluster 4. Their expressions of 374 genes in cluster 3 were highest at 2 wpi and were down-regulated from 4 wpi (Figure 2, Supplemental Table 3). Forty two genes of cluster 4 were up-regulated until 4 wpi and were down-regulated at 8 wpi (Figure 2, Supplemental Table 4). Genes of clusters 3 and 4 were expressed in the stages of nitrogen fixation in fully developed nodule. Functional classification according to classified KOGs database indicated many defense mechanisms genes (2.77%) were identified in these down-regulated genes of clusters 3 and 4 (Figure 1). Although 2.56% of the genes had similarity to ones of function unknown or no similarity to deposited sequences. A full interpretation of the genes have to wait until completion of *L. japonicus* genome annotation. These clusters contained

a number of genes for antioxidant-related proteins such as L-ascorbate oxidase (LjSGA_025815.1), peroxidase (chr1.CM0207.130.r2.m, LjSGA_036806.1), glutathione S-transferase (LjSGA_057970.1, LjSGA_010022.2) and leghemoglobin (chr5.CM0909.860.r2.a) (Figure 2, Supplemental Tables 3 and 4). Becana et al. (2010) showed that antioxidants are important for nodule function. Drought or other types of abiotic stress lead to decreases of antioxidant activities that are associated with nodule senescence (Gogorcena et al. 1997; Pérez Guerra et al. 2010; Porcel et al. 2003; Swaraj et al. 1994). Our data support that decreased activities of antioxidants induced nodule senescence.

To confirm the fidelity of microarray analysis in this study, selected genes were subjected to quantitative reverse transcription PCR (qRT-PCR) according to the manufacturer's protocol (Thermal Cycler Dice Real Time System II; Takara). For qRT-PCR analysis, thirty-

two genes were selected. These specific primers used for qRT-PCR are shown in Supplemental Table 5. The result demonstrated that the expression profiles obtained using the two methods were consistent (Figure 3, Supplemental Figure 1).

To conclude, senescence-associated genes might correspond to trigger degradation of the organs, and conversely preserve and protect cells from environmental stimulus, which leads to aging classified in metabolism, and cellular process. Different expression clusters might reflect the developmental stage of nodule senescence. Especially, genes in cluster 1 appeared specifically in nodule senescence, indicating that these genes can be good markers for the senescence. For many legume crops, nodule senescence coincides with pod filling (Lawn and Brun 1974). Therefore, extending the period of active nitrogen fixation by delaying nodule senescence might have a beneficial effect on crop yield. This microarray data should be helpful in future study of senescence-related genes.

Acknowledgements

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Supplemental Tables and Figure

Supplemental Table 1. Expression profile of 217 genes in cluster 1 during nodule senescence

Supplemental Table 2. Expression profile of 424 genes in cluster 2 during nodule senescence

Supplemental Table 3. Expression profile of 374 genes in cluster 3 during nodule senescence

Supplemental Table 4. Expression profile of 42 genes in cluster 4 during nodule senescence

Supplemental Table 5. Primers list used in this study

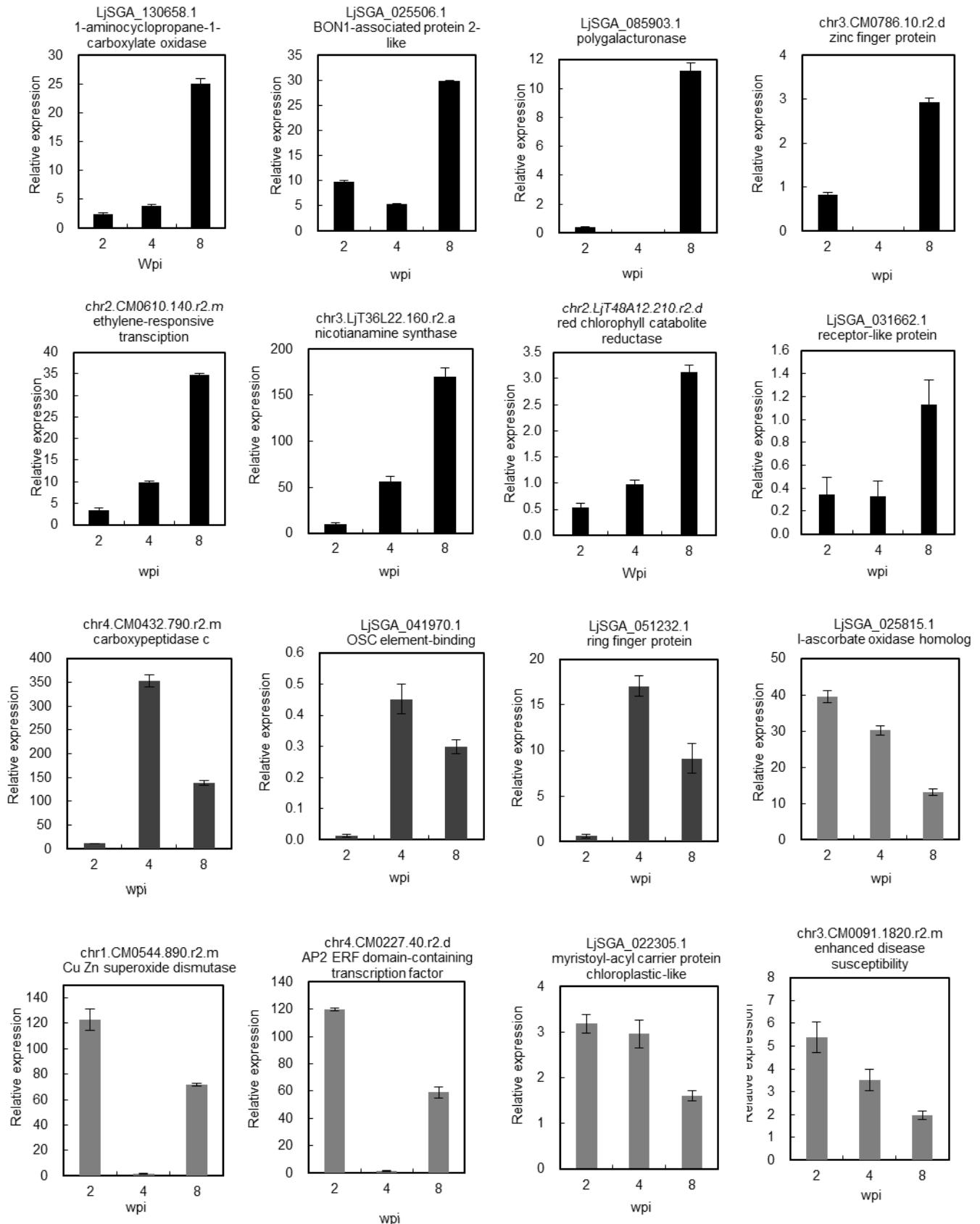
Supplemental Figure 1 Comparison of relative expression levels in the microarray

analysis with qRT-PCR. The other expression profiles classified in cluster 1

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Supplemental Figure1, Chungopast et al.,



Supplemental Table 1. Expression profile of 217 genes in cluster 1 during nodule senescence

ID number	Annotation	2 wpi	4 wpi	8 wpi						
		Average Normalized	SD Normalized	CV (%)	Average Normalized	SD Normalized	CV (%)	Average Normalized	SD Normalized	CV (%)
chr2.CM00191.580>2.m	Nt621 [Lettuce japonicus]	52.296	0.416	10.361	77.871	16.035	20.645	145.909	14.610	9.945
chr1.CM00121.210>2.m	tonoplast intrinsic protein	46.639	1.881	4.033	28.841	0.737	2.555	93.886	2.643	2.815
chr5.CM0456.170>2.d	acidic chitinase	27.983	2.529	9.036	27.087	4.310	15.911	76.881	7.261	9.447
chr1.CM00011.150>2.m	U-box domain-containing protein PR10	24.911	2.342	9.451	27.129	3.239	12.595	92.149	2.539	7.77
LsGA_043683.1	U-box domain-containing protein 20-like	9.800	0.331	3.379	11.513	0.250	2.173	23.093	0.180	0.780
chr5.CM0071.800>2.m	CBL-interacting protein kinase 4	8.498	0.794	9.941	8.418	0.069	0.823	17.988	1.247	6.932
chr2.CM00608.1150>2.m	U-box domain-containing protein 2-like	7.776	0.368	1.170	8.741	0.371	0.422	16.245	1.430	2.35
LsGA_057224.1	trypsin inhibitor	6.664	0.402	5.852	4.383	0.428	0.775	15.333	0.933	6.082
chr1.CM0431.130>2.d	BURP domain-containing protein	6.006	0.189	3.141	5.669	0.503	8.869	25.848	1.452	5.616
chr1.CM00011.150>2.m	U-box domain-containing protein	5.930	0.158	3.109	5.208	0.381	18.691	13.034	0.505	3.675
chr3.LTJ362.22.160>2.m	nicotinamine synthase	3.821	0.372	9.730	4.404	0.654	14.844	60.671	5.328	8.781
chr3.CM0215.240>2.d	ATP-binding cassette	3.409	0.040	1.164	3.855	0.238	8.162	6.835	0.310	4.532
chr2.CM0229.180>2.m	lysine histidine transporter	3.105	0.056	3.005	3.003	0.029	8.926	0.117	0.225	
LsGA_034572.1	probable wrky transcription factor 70-like	2.574	0.058	2.271	1.958	0.010	0.508	6.758	0.170	2.310
chr2.CM0610.140>2.d	ethylene-responsive transcription coactivator-like protein	2.353	0.872	37.034	11.414	4.080	35.573	26.402	9.515	36.038
chr2.CM0262.120>2.m	cytochrome P450	2.292	0.037	1.810	2.085	0.188	6.986	15.908	0.005	6.255
chr1.CM00181.150>2.d	tryptophanyl peptidase-like	2.154	0.009	4.085	1.023	0.098	5.182	0.143	0.247	
LsGA_059352.2	kunitz-type trypsin inhibitor-like protein 2	2.182	0.026	1.212	9.571	0.851	8.890	42.649	1.610	3.774
chr2.CM0246.1390>2.m	soflavone reductase	2.147	0.160	7.441	3.865	0.458	11.844	14.021	0.286	2.040
chr2.CM00011.150>2.m	U-box domain-containing protein	1.997	0.024	2.444	1.022	0.028	4.791	5.795	0.004	0.008
LsGA_030260.1	bont1-associated protein 2-like	1.918	0.150	7.797	1.967	0.223	11.330	4.538	0.428	9.391
chr2.CM0272.460>2.d	lysine histidine transporter	1.886	0.084	4.475	1.786	0.015	0.839	4.025	0.342	8.501
chr2.CM0229.180>2.m	soflavone reductase	1.811	0.112	6.182	2.074	0.207	7.719	12.147	0.465	3.841
chr1.CM0322.190>2.d	soflavone reductase-like	1.752	0.081	4.921	0.868	0.085	7.753	5.537	0.037	0.065
LsGA_034572.1	pathogenesis-related protein 1	1.653	0.038	2.197	0.268	0.001	0.452	9.670	0.059	0.611
chr2.CM0826.310>2.d	brassinosteroid insensitive 1-associated receptor kinase 1	1.583	0.055	3.445	0.953	0.024	20.188	3.223	0.303	9.408
chr1.CM00011.150>2.m	soflavone reductase-like protein 2	1.492	0.009	0.013	0.044	0.024	3.204	0.003	0.000	
LsGA_039552.2	heavy metal transport detoxification domain-containing protein	1.403	0.033	2.318	1.564	0.098	4.194	4.082	0.246	6.032
chr1.CM0375.240>2.d	cytochrome p450	1.376	0.125	9.105	1.954	0.462	23.858	3.916	0.252	6.425
chr2.CM00011.150>2.m	soflavone reductase	1.296	0.007	2.869	1.144	0.032	11.986	3.017	0.187	1.111
chr2.CM00011.370>2.m	cinnamoyl precursor	1.231	0.002	1.033	1.287	0.025	1.906	8.503	0.437	5.135
chr1.CM0088.510>2.d	brassinosteroid insensitive 1-associated receptor kinase 1 precursor	1.188	0.006	0.468	0.997	0.047	4.705	2.520	0.165	6.564
chr2.CM0094.50>2.d	tripetidyl-peptidase-like	1.165	0.016	1.395	0.013	0.013	0.193	2.465	0.034	1.391
chr2.CM0229.180>2.m	probable protein kinase 10<001081941	1.154	0.067	5.119	0.230	0.011	3.691	4.234	0.005	0.223
chr3.CM0279.100>2.m	metalloendopeptidase 1-like	1.134	0.011	1.005	2.286	0.032	1.418	4.535	0.009	0.085
chr4.CM0081.1770>2.m	F-box family protein	1.129	0.073	6.503	1.273	0.089	6.975	3.357	0.269	8.028
chr2.CM00011.150>2.m	soflavone reductase	1.094	0.025	2.570	1.221	0.021	10.171	2.544	0.228	7.777
chr6.CM0584.140>2.d	uncharacterized protein	1.049	0.108	10.291	1.277	0.089	6.979	2.885	0.153	5.314
chr1.CM0108.390>2.m	cytochrome p450	1.048	0.194	18.497	1.429	0.191	13.305	3.288	0.296	9.016
chr2.CM00011.150>2.m	soflavone reductase	1.039	0.009	1.033	0.032	0.009	3.984	1.997	0.184	0.003
LsGA_031246.1	G-branch leucine-rich repeat receptor-like protein kinase RLK1-like	0.872	0.168	19.288	0.577	0.105	18.131	3.376	0.315	9.341
chr1.CM0567.130>2.m	low quality protein	0.860	0.013	1.531	0.739	0.074	10.039	1.897	0.107	5.643
chr5.CM0094.510>2.d	probable leucine-rich repeat receptor-like protein kinase atg5<0380-like	0.799	0.020	2.955	0.828	0.025	2.991	1.803	0.116	6.452
chr2.CM0094.50>2.d	probable leucine-rich repeat receptor-like protein kinase atg2<0250-like	0.746	0.005	4.701	0.260	0.027	14.405	3.030	0.004	0.21
chr1.CM0017.100>2.m	clathrin heavy chain	0.728	0.057	7.770	1.665	0.108	6.463	26.538	2.285	8.609
LsGA_034071.1	clathrin heavy chain	0.644	0.078	12.103	0.378	0.015	13.782	1.456	0.131	9.016
chr1.CM00011.150>2.m	uncharacterized protein	0.634	0.013	1.981	0.043	0.009	9.924	3.381	0.115	2.867
chr1.TA141.110>2.d	Wt-associated receptor kinase-like	0.624	0.012	1.902	0.290	0.010	3.958	1.987	0.015	1.758
chr2.CM0068.560>2.m	ribonuclease t2	0.610	0.062	10.209	0.197	0.012	9.351	1.498	0.116	7.722
chr1.CM0050.450>2.d	cytochrome p450	0.593	0.035	8.249	0.311	0.022	3.335	2.547	0.009	5.757
chr1.CM00011.150>2.m	soflavone reductase	0.519	0.001	1.173	0.040	0.001	7.087	1.143	0.111	2.327
chr2.CM0021.1440>2.d	phospholipase A1-chloroplastic-like	0.500	0.001	1.514	0.499	0.010	2.047	1.802	0.014	0.760
LsGA_030285.1	specific lipid transfer protein	0.489	0.005	1.153	0.262	0.021	7.285	2.640	0.058	2.200
chr1.CM00011.150>2.m	adenosine deaminase-like	0.444	0.006	5.755	0.227	0.025	15.553	4.055	0.166	3.601
chr8.CM0314.230>2.m	bacterial-induced peroxidase	0.444	0.036	8.011	0.146	0.016	11.087	1.426	0.116	8.184
LsGA_030097.2	uncharacterized protein	0.442	0.009	1.215	0.547	0.010	1.876	0.925	0.008	0.819
chr1.CM0040.140>2.m	desoxystyrylase 4-hydroxylase	0.406	0.005	1.195	0.226	0.027	1.201	2.201	0.001	0.215
chr1.CM0404.140>2.m	peroxisomal fatty acid oxidation multifunctional protein	0.390	0.006	1.645	0.542	0.032	5.946	1.090	0.032	2.949
chr3.CM0469.20>2.d	NAC domain-containing protein	0.378	0.007	1.726	0.510	0.028	16.585	0.205	0.019	1.841
chr1.CM00011.150>2.m	soflavone reductase	0.356	0.012	3.279	0.269	0.029	2.547	0.091	0.006	0.055
chr6.CM0573.80>2.d	AP2-like domain-containing transcription factor	0.358	0.024	6.749	0.203	0.020	9.912	0.908	0.037	4.081
chr3.CM0208.430>2.d	late elongated hypocotyl and circadian clock-associated-1-like protein 1	0.347	0.031	8.941	0.117	0.021	2.077	0.718	0.039	5.372
chr1.CM00011.150>2.m	soflavone reductase-like protein	0.346	0.003	9.446	0.133	0.023	2.205	0.015	0.005	0.015
chr1.CM0071.470>2.d	wrky transcription factor	0.338	0.077	22.749	0.614	0.101	18.505	1.130	0.089	9.616
chr5.CM0071.470>2.d	basic 7S globulin 2 precursor small	0.323	0.007	2.233	0.171	0.015	8.973	0.715	0.008	0.488
chr3.CM00011.150>2.m	uncharacterized protein	0.320	0.042	13.047	0.304	0.019	6.087	0.685	0.066	9.640
chr4.CM0006.500>2.m	sulfotransfase 2-like	0.317	0.005	1.650	0.111	0.021	15.203	0.906	0.008	0.033
chr3.CM0404.140>2.d	uncharacterized protein	0.307	0.023	6.425	0.477	0.033	6.944	0.988	0.008	0.018
chr1.CM0404.140>2.m	AP2-like domain-containing transcription factor	0.301	0.001	1.155	0.174	0.009	10.824	0.032	0.003	0.001
chr1.CM0078.300>2.m	potassium channel	0.291	0.007	3.730	0.358	0.038	10.824	0.712	0.071	9.906
LsGA_034572.1	beta-1,3-glucanase	0.289	0.000	2.332	0.107	0.008	9.727	0.670	0.026	3.915
chr1.CM0078.300>2.m	lipid transfer protein	0.282	0.001	7.696	0.337	0.017	5.165	0.990	0.005	0.065
chr5.CM0186.200>2.d	enzyme of the cupin superfamily	0.275	0.012	6.859	0.341	0.021	6.151	7.581	0.008	9.201
chr1.CM0375.180>2.d	uncharacterized protein loc100811015	0.275	0.001	0.953	0.463	0.023	4.946	0.822	0.027	0.712
chr1.CM00011.150>2.m	uncharacterized protein	0.272	0.000	1.016	0.255	0.000	1.504	0.535	0.023	4.288
chr1.TJ1001.50>2.d	alpha-expansin	0.270	0.001	0.910	0.165	0.004	2.650	0.885	0.013	1.487
chr1.CM00011.150>2.m	uncharacterized protein	0.267	0.001	0.944	0.165	0.005	1.935	0.311	0.004	0.025
chr1.CM00011.150>2.m	expoxidase-like protein	0.265	0.005	3.039	0.265	0.037	12.408	0.776	0.005	3.144
chr1.CM0080.260>2.d	uncharacterized protein	0.262	0.005	3.275	0.174	0.012	10.008	0.906	0.008	3.144
chr1.CM00011.150>2.m	expoxidase-like protein	0.259	0.001	1.140	0.264	0.003	4.100	0.304	0.005	0.028
chr3.CM0232.340>2.m	beta-amin synthase	0.253	0.001	8.722	0.111	0.002	1.358	0.258	0.018	7.717
chr3.CM0261.600>2.m	receptor protein kinase clavata1	0.253	0.006	11.339	0.066	0.001	0.998	0.182	0.014	7.815
chr1.CM0003.290>2.d	Ca2+ importer calmodulin exchanger	0.252	0.004	1.027	0.058	0.004	5.048	0.246	0.009	3.579
chr1.CM0003.290>2.d	uncharacterized protein	0.252	0.008	3.207	0.079					

LjT13F18.100-2.d	no hit
LjSGA_00000000.2.d	high affinity inorganic phosphate transporter
dhv-3.0728.390-2.d	tropomodulin homolog at10740-like
LjSGA_00831.1	wall-associated receptor kinase 2-like
chr2.CM0008.40-2.d	carbonic anhydrase
LjSGA_00000000.2.d	uncharacterized protein
dhv-3.CM0108.400-2.d	transmembrane peptidyl-ubiquinone oxidoreductase
LjSGA_004604.1	protein SRG1-like
LjSGA_001726.2	glutathione-NBS-LRR resistance protein
LjSGA_00000000.2.d	glutathione amidotransferase subunit A
LjSGA_001631.1	uncharacterized protein
LjSGA_003080.2	uncharacterized protein
dhv-2.CM0124.130-2.d	heat shock protein
dhv-4.LT0082.190-2.d	RCC1 and BTB domain-containing protein
LjSGA_003126.1	ATP efflux family protein 8-like
LjSGA_00000000.2.d	Cytokine receptor-like protein
LjSGA_008259.3	beta-glucan-binding protein
LjSGA_004269.2	ATP binding protein
dhv-3.CM0008.40-2.d	uncharacterized protein
chr8.CM0338.460-2.d	uncharacterized protein
LjSGA_004251.1	sarcosine oxidase
LjSGA_014861.1	expansin precursor
dhv-2.CM0141.1450-2.d	ABC transporter B family member
dhv-2.MT0154.7.30-2.m	disease resistance response protein 206-like

Supplemental Table 2. Expression profile of 424 genes in cluster 2 during nodule senescence

ID number	Annotation	2 esp			4 esp			8 esp			
		Average	Normalized	SD	Normalized	CV (%)	Average	Normalized	SD	Normalized	CV (%)
chr1.CM0195.250-2.m	cysteine protease 15A-like	30.063	1.321	4.119	97.327	2.516	2.585	68.842	2.977	4.454	6.815
chr1.CM0008.970-2.m	amino acid transporter PTR3-a	30.030	3.181	10.695	110.950	8.459	7.642	65.695	2.653	5.033	5.772
chr1.CM0008.970-2.m	uncharacterized protein	21.634	0.030	0.142	10.29	1.155	1.155	54.469	1.021	1.209	1.15
chr3.CM0135.890-2.m	cyclophilin	24.452	1.173	4.796	41.991	0.192	0.458	52.823	1.251	2.388	8.517
LjSGA_015484.1	cysteine protease	0.023	0.004	15.518	0.227	0.005	0.075	0.285	0.024	0.004	0.024
dhv-3.CM0108.400-2.d	subtilisin-like protease SDD1-like	20.564	0.004	4.460	30.101	0.001	0.011	50.033	0.031	2.233	0.024
LjSGA_002721.1	peptidase S1-like	15.811	1.524	9.636	30.233	1.528	5.053	47.706	0.070	1.001	0.021
chr1.CM0270.120-2.d	UNC93-like protein	11.243	0.661	5.877	30.391	1.732	5.700	25.932	1.658	6.392	0.024
chr1.CM0017.470-2.d	transaldolase	11.115	0.215	10.739	19.464	2.518	12.934	25.809	2.436	9.437	0.024
LjSGA_00000000.2.d	uncharacterized protein	11.065	0.006	5.477	15.263	1.532	2.750	21.707	1.170	4.444	0.024
chr4.CM0432.790-2.m	carboxypeptidase C	2.882	0.036	1.330	29.320	1.487	5.071	23.293	0.418	1.796	0.024
LjSGA_016179.1	cysteine symmetric	9.101	1.159	1.742	16.159	0.491	3.038	23.160	0.119	0.513	0.024
dhv-3.CM0108.400-2.d	type 2 metallophloioin	9.947	0.116	1.133	15.559	0.259	3.705	20.010	0.156	0.441	0.024
LjSGA_019242.2	type 2 metallophloioin	5.134	0.103	2.005	15.755	0.081	0.384	21.900	0.380	1.737	0.024
chr1.CM0591.60-2.m	no hit	3.764	0.032	0.849	27.809	0.497	1.799	19.768	0.567	2.866	0.024
LjSGA_00000000.2.d	type 2 metallophloioin	0.701	0.073	0.030	10.501	0.001	0.034	16.252	1.137	3.037	0.024
chr4.CM0165.230-2.d	calmodulin-like protein	4.398	0.193	4.396	14.110	0.662	4.693	14.072	0.428	3.027	0.024
chr4.CM0075.30-2.m	uncharacterized protein	5.517	0.291	5.271	9.936	0.290	1.290	14.037	0.835	5.948	0.024
chr3.CM0160.370-2.m	uncharacterized protein	5.099	0.061	1.189	13.330	0.255	1.910	13.479	0.545	4.040	0.024
dhv-3.CM0108.400-2.d	14 Dsproline-rich protein	5.290	0.164	3.110	14.149	0.191	1.091	13.381	0.382	3.556	0.024
LjSGA_019308.1	uncharacterized protein	5.938	0.103	1.818	16.181	0.396	2.445	13.325	0.364	2.730	0.024
chr3.CM0160.360-2.m	14 Dsproline-rich protein	5.274	0.091	1.716	12.902	0.585	1.453	13.297	0.772	5.806	0.024
chr4.CM0075.30-2.m	14 Dsproline-rich protein	4.530	0.077	0.998	12.050	0.141	1.056	12.955	0.256	3.022	0.024
chr5.CM0698.540-2.d	copper chaperone	2.413	0.103	4.282	27.586	1.856	6.728	11.984	0.702	5.860	0.024
chr3.CM0127.770-2.d	small heat shock protein	1.670	0.125	7.484	4.705	0.678	14.310	10.519	0.441	4.190	0.024
chr5.CM0008.970-2.m	uncharacterized protein	1.253	0.006	0.686	9.338	0.238	4.737	9.824	0.533	5.934	0.024
chr5.CM0244.350-2.d	Zn Cd Prp-like ATPase	4.193	0.140	3.333	15.552	0.239	6.077	9.938	0.408	4.001	0.024
chr1.CM0718.490-2.m	glycine-rich RNA-binding protein	2.733	0.244	7.470	13.322	1.564	11.741	8.918	0.753	8.445	0.024
chr3.CM0416.890-2.d	uncharacterized protein	4.207	0.384	9.372	13.466	1.490	11.067	8.564	0.834	9.743	0.024
chr4.CM0075.30-2.m	uncharacterized protein	4.002	0.036	1.586	10.000	0.201	0.327	9.331	0.323	3.051	0.024
chr5.CM0008.970-2.m	intracellular chloride channel	3.289	0.020	0.614	11.810	1.256	10.545	8.300	0.400	4.820	0.024
chr6.CM0245.590-2.d	serine acetyltransferase mitochondrial	1.185	0.007	0.623	12.167	0.354	2.095	8.095	0.319	3.942	0.024
LjSGA_00000000.2.d	auxin-binding protein Sng1-like	1.105	0.083	7.502	8.363	0.245	10.559	8.000	0.580	7.255	0.024
LjSGA_00000000.2.d	auxin-binding protein Sng1-like	3.158	0.007	2.025	12.032	0.258	2.405	7.296	0.102	3.933	0.024
LjSGA_014322.1	acyl-coenzyme A oxidase peroxisomal-like	2.319	0.068	4.225	8.588	0.453	5.281	7.123	0.451	6.338	0.024
chr1.CM0008.970-2.m	small heat shock protein	3.026	0.501	16.588	16.588	0.106	18.449	15.573	0.573	8.612	0.024
chr5.CM0008.970-2.m	uncharacterized protein	2.451	0.004	3.449	14.042	0.258	2.155	6.414	0.312	3.117	0.024
chr3.CM0116.300-2.m	divalent 3'-phosphate acyltransferase	3.371	0.970	28.781	2.197	0.664	30.204	6.058	1.755	28.973	0.024
LjSGA_002398.1	homoeobox zipper protein HATT2-like	1.901	0.077	4.034	3.384	0.135	3.999	5.682	0.071	1.259	0.024
chr1.CM0008.970-2.m	zinc finger protein zat1-like	1.032	0.110	8.113	12.134	0.194	3.020	5.251	0.300	3.020	0.024
chr3.CM0238.90-2.m	zinc finger protein zat1-like	2.078	0.028	1.239	3.218	0.124	3.854	4.901	0.168	0.334	0.024
chr4.CM0432.950-2.d	RNA polymerase-associated protein RTTF1 homolog	2.195	0.320	14.567	6.096	1.196	19.624	4.700	0.419	4.457	0.024
chr5.LT013404.40-2.d	ATL23-like	1.891	0.034	1.795	10.328	0.009	0.009	1.772	0.034	2.322	0.024
LjSGA_00000000.2.d	uncharacterized protein	0.327	0.009	5.491	3.039	0.141	3.075	4.004	0.337	3.111	0.024
chr3.LT0706.80-2.m	uncharacterized protein	1.595	0.049	3.066	3.309	0.149	4.496	3.906	0.063	1.624	0.024
chr3.CM0155.30-2.d	heat shock protein	0.493	0.050	10.133	2.405	0.225	9.400	3.815	0.274	7.180	0.024
chr5.CM0008.970-2.m	F-actin-regulated protein	1.498	0.031	2.124	10.219	0.155	1.041	3.791	0.245	4.841	0.024
LjSGA_017410.70-2.m	regulator of ozone expressed	1.504	0.004	2.073	2.988	0.168	5.891	3.740	0.100	2.963	0.024
LjSGA_00000000.2.d	chaperone protein dnd1	1.433	0.022	1.200	6.641	0.468	6.783	3.634	0.034	4.032	0.024
chr1.CM0017.280-2.m	uncharacterized protein	1.771	0.046	2.677	10.230	0.143	3.043	3.377	0.111	4.771	0.024
chr5.CM0077.520-2.d	no hit	0.752	0.028	3.755	5.371	0.267	3.467	2.663	0.263	2.443	0.024
LjSGA_007081.1	alpha/beta carboxyphosphatase	1.740	0.105	4.034	1.334	0.135	3.999	5.250	0.300	2.223	0.024
chr5.CM0008.970-2.m	alpha/beta carboxyphosphatase	1.005	0.009	1.229	1.229	0.160	1.219	2.772	0.034	3.335	0.024
chr5.CM0203.20-2.d	uncharacterized protein	0.914	0.024	2.653	3.071	0.225	2.739	2.771	0.274	8.890	0.024
chr5.CM0052.410-2.d	no hit	0.732	0.081	11.134	2.506	0.016	1.620	2.717	0.180	6.608	0.024
chr5.CM0008.970-2.m	uncharacterized protein	1.227	0.005	8.605	10.009	0.003	4.152	2.819	0.175	6.698	0.024
chr5.CM0059.500-2.m	uncharacterized protein	0.736	0.031	3.029	1.014	0.141	2.543	2.046	0.108	3.038	0.024
LjSGA_005949.1	DNA-binding protein escala-like	1.068	0.079	7.354	2.013	0.151	7.485	2.407	0.188	7.711	0.024
chr6.CM0314.680-2.m	vacuolar iron transporter-like protein	0.743	0.041	5.545	3.149	0.194	6.150	2.386	0.084	3.537	0.024
chr5.CM0008.970-2.m	uncharacterized protein	0.698	0.006	2.240	10.203	0.003	0.267	2.127	0.086	4.046	0.024
LjSGA_022311.1	auxin-induced protein Sng4-like	1.123	0.134	11.958	2.598	0.340	13.308	3.206	0.146	6.323	0.024
LjSGA_112841.1	aminophospholipid atpase	1.094	0.016	1.506	2.236	0.018	0.827	2.250	0.024	1.067	0.024
LjSGA_004497.1	proteins	0.585	0.039	3.276	4.845	0.169	4.845	2.249	0.030	1.201	0.024
LjSGA_118123.1	uncharacterized protein	0.637	0.014	2.711	0.911	0.043	4.761	2.226	0.031	1.389	0.024
chr5.CM0040.640-2.d	kinase-like protein	0.574	0.003	3.042	0.926	0.020	3.477	1.198	0.138	1.575	0.024
chr5.CM0458.180-2.d	kinase-like protein	0.171	0.006	3.446	0.948	0.028	3.446	1.153	0.135	1.553	0.024
chr5.CM0057.70-2.d	serine-threonine kinase	0.171	0.006	3.449	0.948	0.028	3.446	1.153	0.135	1.553	0.024
LjSGA_00000000.2.d	uncharacterized protein	0.817	0.074	9.062	2.807	0.249	8.882	1.860	0.086	5.160	0.024
chr5.CM1687.270-2.m	leucine-rich repeat family protein	0.652	0.058	8.684	0.791	0.108	13.845	1.857	0.137	8.255	0.024
chr1.LT1383.19-20.2.d	Vamp-like protein	0.617	0.002	1.330	1.330	0.155	1.030	1.030	0.03		

chr5.LT29P04.10.z.d	uncharacterized protein	0.227	0.010	4.555	0.628	0.014	2.288	0.684	0.028	4.131	
chr1.CM001.240.z.m	auxin-induced protein Snp1-like	0.153	0.008	4.910	0.537	0.015	2.745	0.682	0.039	5.653	
chr1.LT29L02.2.d	auxin-induced protein Snp1-like	0.010	0.002	9.322	0.523	0.020	14.237	0.659	0.046	5.446	
chr2.CM017.120.z.d	cinnamyl alcohol dehydrogenase	0.245	0.012	4.767	0.445	0.039	8.667	0.658	0.054	8.247	
LsGA_0324031	protein transport protein sec31	0.291	0.008	2.741	0.431	0.011	2.663	0.613	0.040	6.564	
chr1.CM001.240.z.d	phox domain-containing protein	0.294	0.005	11.889	0.449	0.026	17.142	0.611	0.036	9.138	
chr1.CM0012.220.z.d	uncharacterized protein	0.040	0.004	8.479	0.566	0.014	2.429	0.600	0.029	7.986	
chr6.CM053.140.z.m	topless-related protein 3-like	0.276	0.013	4.651	0.508	0.032	6.292	0.585	0.043	7.293	
chr4.CM031.170.z.d	uncharacterized protein	0.165	0.002	0.000	0.126	0.050	0.025	3.912	0.584	0.020	3.475
chr2.CM009.240.z.m	uncharacterized protein loc10080669	0.140	0.002	1.736	0.500	0.010	3.392	0.570	0.024	3.27	
chr2.CM012.140.z.m	phot-like protein	0.286	0.022	7.528	0.465	0.024	5.234	0.574	0.025	4.426	
chr2.CM012.140.z.m	wax2-like	0.061	0.005	8.632	0.626	0.042	6.746	0.570	0.003	5.951	
LsGA_015817.1	uncharacterized protein	0.246	0.007	10.774	0.553	0.047	4.035	0.590	0.015	4.45	
LsGA_006701.1	transcription factor UNE10-like	0.193	0.001	0.767	0.495	0.005	1.107	0.554	0.004	0.772	
chr4.CM001.240.z.d	probable purine permease 11-like	0.255	0.002	0.923	0.501	0.006	1.235	0.553	0.015	2.775	
chr1.CM001.230.z.d	phosphoribosyloaminoimidazole-succinocarboxamide synthase	0.246	0.005	10.930	0.529	0.029	13.005	0.524	0.028	4.955	
LsGA_1LT31L11.70.z.d	F-box protein ORE9-like	0.212	0.005	2.303	0.677	0.036	5.317	0.519	0.003	0.759	
LsGA_1LT31N08.80.z.m	F-box protein	0.164	0.014	8.777	0.687	0.032	4.687	0.504	0.038	7.520	
chr1.CM001.240.z.d	kinase finger domain-containing protein 30-like	0.200	0.005	12.404	0.523	0.029	20.040	0.450	0.036	5.56	
chr3.CM150.240.z.m	lysine/arginine-rich nucleotidyl transferase	0.178	0.015	8.377	0.400	0.060	15.018	0.479	0.018	3.258	
chr5.CM129.40.z.m	transcription factor TCP15	0.160	0.013	7.888	0.654	0.019	2.928	0.456	0.016	3.444	
LsGA_015818.1	uncharacterized protein	0.143	0.004	2.558	0.555	0.021	8.660	0.452	0.005	1.215	
chr2.CM004.150.z.d	aminoacyl-tRNA ligase	0.212	0.006	2.448	0.373	0.021	8.380	0.445	0.009	0.779	
LsGA_034647.1	agent domain-containing protein expressed	0.172	0.012	6.715	0.262	0.024	8.270	0.442	0.019	4.353	
LsGA_014378.1	alpha/beta-hydrolase domain-containing protein	0.177	0.015	8.501	0.411	0.025	5.637	0.439	0.021	4.687	
LsGA_017020.1	ethylene response factor 1-like	0.201	0.000	9.909	0.339	0.023	9.889	0.430	0.020	4.444	
chr5.CM055.670.z.d	transcription factor	0.144	0.022	15.384	0.459	0.018	3.909	0.427	0.020	4.703	
chr2.LT16L140.70.z.d	receptor-like protein loc1019471-like	0.091	0.014	14.673	0.746	0.043	4.362	0.424	0.028	6.512	
chr4.CM009.630.z.d	lysine/arginine-rich nucleotidyl transferase	0.096	0.010	10.805	0.288	0.015	5.234	0.414	0.030	7.142	
LsGA_020918.1	cytosolic alpha-amylase	0.035	0.002	6.912	0.174	0.009	4.664	0.412	0.020	4.980	
chr1.CM164.10.z.d	La protein	0.174	0.011	6.288	0.574	0.027	4.750	0.409	0.021	5.037	
chr2.CM001.240.z.m	uncharacterized protein Smg1-like	0.030	0.003	1.101	0.205	0.025	3.325	0.404	0.026	3.224	
chr1.CM063.180.z.d	SPB domain-containing membrane protein at4g22990-like isoform I	0.198	0.003	3.180	0.727	0.021	4.205	0.399	0.028	7.081	
LsGA_02284.300.z.m	ATPase family member 20-like	0.014	0.003	19.116	0.404	0.059	14.643	0.395	0.039	9.645	
chr1.CM001.230.z.d	uncharacterized protein 8 second largest subunit	0.132	0.002	1.405	0.203	0.020	4.085	0.391	0.010	4.584	
LsGA_017232.1	uncharacterized protein	0.158	0.009	5.598	0.440	0.001	0.314	0.391	0.030	7.782	
chr5.CM041.340.z.d	ethylene responsive transcription factor 1b-like	0.059	0.010	16.379	0.239	0.000	0.016	0.387	0.030	7.797	
LsGA_025450.1	uncharacterized protein	0.120	0.002	6.174	0.332	0.000	3.060	0.385	0.025	9.115	
LsGA_017021.1	ethylene responsive transcription factor 1b-like	0.157	0.003	1.004	0.247	0.017	0.353	0.384	0.024	4.952	
chr2.CM0021.340.z.m	uncharacterized protein	0.174	0.005	2.710	0.546	0.038	6.960	0.384	0.013	3.416	
LsGA_1LT1516.1	soul heme-binding family protein	0.091	0.001	1.005	0.398	0.009	1.911	0.383	0.009	2.372	
chr2.CM001.240.z.d	uncharacterized protein	0.171	0.006	3.359	0.261	0.017	7.387	0.381	0.021	5.58	
chr3.CM011.150.z.d	no hit	0.152	0.015	9.711	0.458	0.023	4.984	0.381	0.003	0.801	
LsGA_023284.1	B-Block binding subunits of TFBIC	0.096	0.000	0.084	0.326	0.018	5.563	0.377	0.005	1.211	
LsGA_017022.1	aminoacyl-tRNA ligase ATPase	0.175	0.013	7.550	0.333	0.020	4.165	0.377	0.012	3.30	
LsGA_033442.1	calmodulin-like protein	0.061	0.008	12.259	0.459	0.032	7.072	0.374	0.017	4.506	
chr2.CM168.300.z.d	galactomannan galactosyltransferase	0.152	0.014	9.279	0.312	0.005	1.694	0.371	0.021	6.840	
chr5.CM010.180.z.d	kinase family protein	0.162	0.004	2.727	0.334	0.003	0.896	0.368	0.012	3.383	
LsGA_017023.1	uncharacterized protein	0.111	0.008	7.689	0.338	0.020	3.203	0.359	0.027	4.447	
chr5.CM020.3040.2.z.m	zinc finger protein 4	0.072	0.005	10.642	0.596	0.051	4.788	0.357	0.019	5.312	
LsGA_026288.1	s-adenyly-L-methionine-dependent methyltransferase-like protein	0.169	0.016	9.405	0.253	0.009	3.572	0.355	0.008	1.241	
LsGA_017024.1	uncharacterized protein	0.110	0.001	1.005	0.388	0.009	0.421	0.350	0.004	0.951	
chr1.CM025.990.z.m	peptide transporter PTR3A	0.026	0.008	24.323	0.545	0.031	5.640	0.348	0.005	1.352	
chr4.CM001.250.z.d	uncharacterized protein	0.171	0.001	0.377	0.346	0.013	3.836	0.347	0.014	4.150	
LsGA_006341.1	LON protease inhibitor-like protein	0.142	0.003	9.313	0.274	0.027	9.835	0.345	0.020	5.769	
chr1.CM010.180.z.d	aminoacyl-tRNA ligase splicing factor	0.168	0.005	3.208	0.311	0.002	0.479	0.343	0.008	0.553	
chr5.CM050.500.z.d	one helix protein 2 protein	0.166	0.007	4.206	0.587	0.015	2.514	0.341	0.028	7.598	
chr5.CM045.340.z.d	ATP-directed RNA polymerase III subunit RPCS	0.114	0.002	2.083	0.395	0.021	2.821	0.341	0.032	9.524	
LsGA_010765.1	ATP-binding cassette transporter	0.125	0.003	2.433	0.341	0.001	0.558	0.330	0.003	0.951	
LsGA_029395.1	cysteine-rich repeat protein	0.165	0.000	1.233	0.567	0.008	1.281	0.337	0.000	1.798	
LsGA_025450.1	WD40 repeat compass complex protein	0.136	0.003	2.728	0.760	0.010	1.360	0.334	0.024	5.877	
LsGA_026342.1	acetyl-CoA acetyltransferase	0.137	0.002	1.809	0.316	0.011	3.329	0.296	0.011	3.641	
chr4.CM044.1342.2	nucleotide-diphospho-sugar transferase	0.061	0.005	8.406	0.363	0.008	2.606	0.294	0.009	3.110	
chr5.CM010.180.z.d	uncharacterized protein	0.094	0.012	12.903	0.521	0.020	7.097	0.326	0.025	4.644	
chr4.CM126.150.z.m	beta-glucuronidase-like protein	0.133	0.010	7.682	0.278	0.018	6.358	0.289	0.001	0.377	
chr4.CM187.150.z.d	uncharacterized protein	0.018	0.001	7.465	0.151	0.022	1.227	0.288	0.000	0.946	
chr1.CM030.180.z.d	poly(A)-dependent gene nemf1-like	0.141	0.002	15.592	0.524	0.020	2.013	0.315	0.029	4.204	
chr2.CM022.890.z.d	histidine kinase osmosensor protein	0.082	0.001	1.461	0.293	0.007	2.370	0.313	0.018	5.827	
LsGA_013005.1	uncharacterized protein	0.095	0.041	42.710	0.199	0.003	1.276	0.311	0.002	0.632	
chr2.CM027.890.z.d	histidine kinase osmosensor protein	0.118	0.005	6.613	0.299	0.010	3.289	0.297	0.017	4.449	
LsGA_047898.1	cytochrome P450 monooxygenase cytP285	0.118	0.007	6.319	0.265	0.007	2.262	0.297	0.027	9.014	
chr1.CM0140.140.z.d	ATP-binding cassette transporter	0.096	0.001	0.701	0.255	0.003	7.447	0.279	0.024	6.240	
LsGA_010410.1	leucine zipper protein	0.094	0.002	1.991	0.216	0.001	2.391	0.279	0.003	0.609	
chr1.CM023.70.z.d	Wry transcription factor 50	0.089	0.004	1.004	0.247	0.008	2.894	0.241	0.005	2.216	
chr2.CM036.220.z.d	leucine zipper-domain-like protein	0.088	0.010	17.651	0.314	0.005	2.547	0.234	0.011	4.659	
LsGA_027482.1	leucine zipper-domain-like protein	0.084	0.003	3.078	0.358	0.009	10.828	0.233	0.014	6.122	
chr1.CM017.190.z.d	wall-associated receptor kinase-like 14-like	0.108	0.005	4.332	0.320	0.009	4.409	0.231	0.009	3.723	
chr2.CM023.251.z.d	uncharacterized protein kinase	0.107	0.006	5.443	0.219	0.001	0.270	0.227	0.013	5.584	
chr5.CM032.240.z.d	uncharacterized protein kinase	0.072	0.011	15.707	0.232	0.014	5.892	0.203	0.008	4.115	
LsGA_030971.1	ADP-ribosylation factor GTPase-activating protein AGD14	0.092	0.007	4.466	0.322	0.005	6.765	0.201	0.008	3.184	
chr5.CM001.200.z.d	protein zdf7	0.024	0.003	13.926	0.264	0.014	4.719	0.197	0.020	2.74	
LsGA_014246.1	proline-rich protein 1-like	0.085	0.011	13.468	0.159	0.007	4.201	0.198	0.011	5.725	
chr1.CM161.160.z.d	thioredoxin reductase 1 protein	0.039	0.002	5.128	0.189	0.003	7.039	0.197	0.007	3.680	
LsGA_039118.1	thioredoxin thiol reductase BSL2-like	0.088	0.001	18.414	0.285	0.003	5.096	0.196	0.003	2.024	
chr1.CM031.210.z.d	uncharacterized protein	0.039	0.002	18.354	0.147	0.008	2.091	0.191	0.002	0.978	
chr1.CM143.120.z.d	transportin 1	0.069	0.003	4.447	0.179	0.011	6.068	0.141	0.000	0.257	
chr4.CM006.520.z.d	alpha/beta hydrolase-like protein	0.055	0.002	3.784	0.140	0.010	6.874	0.141	0.009	0.257	
chr1.CM001.200.z.m	alpha/beta hydrolase-like protein ZAT5-like	0.0									

chr1.CM0113.220.r2.m	pentapeptide repeat-containing	0.027	0.004	14.723	0.068	0.001	1.130	0.073	0.003	4.455
LsGA_0360181.120.r2.m	membrane anchored calmodulin-fold protein 2	0.015	0.001	6.603	0.042	0.001	2.835	0.073	0.001	1.589
chr4.CM0224.90.r2.m	uncharacterized protein	0.030	0.003	7.147	0.054	0.007	11.348	0.072	0.001	1.900
chr2.CM0168.310.r2.d	galactosmannan galactosyltransferase	0.025	0.010	38.856	0.079	0.006	7.494	0.072	0.007	9.432
LsGA_0360182.120.r2.m	uncharacterized protein	0.023	0.004	15.880	0.047	0.001	1.739	0.069	0.004	5.486
chr4.CM0041.990.r2.m	uncharacterized protein	0.018	0.003	9.537	0.048	0.000	10.937	0.069	0.002	3.231
LsGA_0360171.1	uncharacterized protein	0.030	0.002	19.226	0.061	0.008	13.250	0.069	0.000	4.012
LsGA_0452071.1	uncharacterized protein	0.015	0.004	27.395	0.069	0.001	1.305	0.067	0.006	0.523
chr1.CM0113.220.r2.d	amino acid transporter	0.032	0.003	8.558	0.071	0.007	10.025	0.067	0.002	2.081
LsGA_0174112.1	galacturonosyltransferase	0.029	0.001	4.733	0.059	0.000	0.124	0.066	0.000	8.811
LsGA_033491.1	transducin family prote	0.032	0.001	3.097	0.069	0.001	0.756	0.066	0.006	9.685
LsGA_0360183.1	cytosolic nucleotide-binding protein kinase	0.030	0.006	20.599	0.073	0.003	4.353	0.066	0.003	3.441
LsGA_0288641.2	ankyrin repeat-containing	0.024	0.000	1.387	0.072	0.015	21.338	0.065	0.001	2.163
chr5.CM0018.70.r2.m	cytosolic purine 5'-nucleotidase-like	0.014	0.000	2.528	0.035	0.011	31.858	0.064	0.002	3.217
CM0018.70.r2.m	inorganic phosphate transporter 2-1	0.030	0.001	3.105	0.042	0.000	6.838	0.064	0.000	0.439
LsGA_0360179.1	uncharacterized protein	0.021	0.002	11.650	0.062	0.003	4.688	0.063	0.003	1.681
LsGA_0193301.1	RNA-dependent RNA polymerase	0.022	0.001	5.574	0.037	0.002	4.145	0.060	0.000	5.401
LsGA_0293931.1	mystein-like protein	0.024	0.000	8.302	0.061	0.014	23.090	0.060	0.000	3.863
LsGA_0360185.1	uncharacterized protein	0.026	0.002	3.241	0.056	0.000	0.147	0.055	0.000	0.590
LsGA_0134441.1	basic helix-loop-helix protein bHLH	0.024	0.002	8.725	0.053	0.008	15.143	0.058	0.002	3.533
LsGA_0459581.1	probable protein phosphatase 2C-like	0.028	0.007	24.795	0.049	0.002	4.726	0.057	0.005	8.954
LsGA_0360173.1	xylosyltransferase-like	0.025	0.001	7.531	0.050	0.000	2.055	0.056	0.000	4.035
chr5.CM0188.70.r2.m	uncharacterized protein	0.023	0.001	5.188	0.047	0.010	20.620	0.056	0.003	3.475
chr5.CM015881.1	kinase-like protein	0.018	0.004	22.327	0.058	0.002	5.391	0.054	0.005	9.843
LsGA_068691.1	drab homolog subfamily C member 13-like	0.021	0.001	13.738	0.037	0.012	32.115	0.052	0.004	6.731
LsGA_0473481.1	uncharacterized protein	0.026	0.000	12.446	0.045	0.005	12.614	0.052	0.005	9.083
LsGA_0360181.1	protein kinase C-like	0.020	0.000	24.468	0.044	0.013	1.038	0.052	0.004	1.683
LsGA_0484071.1	AAA ATPase containing wimberley factor type a domain-containing protein	0.016	0.000	2.437	0.044	0.002	5.185	0.051	0.000	0.630
chr4.LB1707.20.r2.d	TIR domain proteins	0.017	0.002	12.475	0.131	0.000	7.071	0.050	0.004	7.814
chr5.CM0008.110.r2.m	no hit	0.024	0.000	13.760	0.039	0.011	11.172	0.049	0.000	2.025
chr2.CM0028.780.2.m	NBS-IRNP domain	0.023	0.000	1.543	0.046	0.002	5.077	0.049	0.000	7.227
LsGA_0360180.1	leucine-rich repeats protein 15f5	0.014	0.001	6.606	0.049	0.002	3.130	0.047	0.001	2.126
LsGA_076871.1	B-Block binding subunit of TFBIC	0.023	0.002	9.757	0.047	0.007	14.128	0.047	0.002	3.951
LsGA_075588.1	uncharacterized protein	0.023	0.005	20.546	0.045	0.005	11.326	0.047	0.005	9.671
chr5.CM0019.240.r2.m	pentapeptide repeat-containing protein	0.019	0.001	3.069	0.130	0.000	4.950	0.046	0.004	9.603
chr5.CM0101.20.r2.d	protein kinase C-like protein	0.021	0.000	12.431	0.028	0.013	1.117	0.041	0.001	1.37
LsGA_0484071.1	AMP deaminase	0.020	0.002	8.624	0.074	0.002	2.869	0.045	0.000	0.325
LsGA_041425.1	squamous papilloma-binding protein 7	0.021	0.001	7.272	0.054	0.000	27.141	0.045	0.000	1.414
chr5.CM0008.170.r2.d	probable membrane protein	0.022	0.002	3.745	0.046	0.005	10.937	0.045	0.003	4.447
LsGA_093321.1	ABC transporter D family member	0.022	0.001	2.328	0.042	0.007	17.359	0.044	0.002	4.896
LsGA_0154521.1	triflex transcription factor GT-2-like	0.014	0.001	10.338	0.057	0.003	4.572	0.043	0.002	4.884
chr5.CM0120.240.r2.d	PAP-domain containing protein	0.019	0.000	10.799	0.059	0.000	8.915	0.043	0.000	3.867
LsGA_068771.1	HAL2-like	0.019	0.002	9.330	0.030	0.001	3.442	0.042	0.004	9.910
LsGA_108877.1	sensitive to freezing 6 protein	0.016	0.001	5.372	0.037	0.002	5.301	0.042	0.002	3.685
chr3.CM0129.170.r2.d	DNA mismatch repair protein mhl	0.019	0.003	14.778	0.075	0.000	0.380	0.042	0.000	0.862
chr5.CM0590.140.r2.m	uncharacterized protein loc1007861119	0.017	0.000	14.231	0.028	0.000	5.117	0.041	0.001	2.052
chr1.CM0088.130.r2.m	presequence protease 2	0.017	0.000	8.827	0.046	0.003	4.900	0.039	0.000	8.721
chr5.CM0120.170.r2.m	desmosome-associated protein	0.016	0.002	10.521	0.046	0.000	14.955	0.039	0.001	2.774
LsGA_0360181.1	phosphorylase/butyryl/carnitine synthase	0.019	0.004	19.754	0.050	0.002	4.889	0.038	0.003	7.369
LsGA_0309571.1	probable Imr receptor-like serine/threonine-protein kinase RKF3-like	0.018	0.001	5.943	0.026	0.004	16.430	0.038	0.000	1.061
LsGA_0360184.1	two-component response regulator ARR11-like	0.015	0.003	18.815	0.046	0.005	14.602	0.037	0.002	4.167
chr5.CM0173.120.r2.d	ATP-binding cassette protein	0.017	0.000	16.704	0.040	0.005	13.314	0.035	0.002	3.553
chr6.CM0383.550.r2.d	atG2103-like protein	0.015	0.001	8.450	0.048	0.005	9.866	0.034	0.001	4.349
chr1.CM0113.150.r2.d	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily a member 3-like	0.015	0.001	3.882	0.041	0.002	3.935	0.032	0.002	6.684
LsGA_048592.1	parthenocyste kinase 2-like	0.013	0.001	3.931	0.024	0.002	8.914	0.030	0.002	6.086

Supplemental Table 3. Expression profile of 374 genes in cluster 3 during nodule senescence

ID number	Annotation	Z wpc	Average Normalized	SD Normalized	CV (%)	wpc	Average Normalized	SD Normalized	CV (%)	wpc
chr1.CM0104.2710.2.r2.m	arabinogalactan peptide 14	15.719	8.158	3.918	77.180	0.255	6.576	28.978	3.538	4.455
chr1.CM0227.150.r2.m	peptidyl peptide transporter atg24060-like	70.287	2.572	3.680	46.267	3.043	6.576	28.978	3.538	4.455
chr1.CM0227.150.r2.m	EGF-like domain	57.041	2.595	6.909	34.938	3.043	6.576	24.445	4.457	4.455
chr1.CM0227.402.r2.m	AP2/erf domain-containing transcription factor	41.622	2.690	6.464	25.978	0.198	0.783	7.872	0.236	3.003
chr1.CM0044.890.r2.m	cu zu superoxide dismutase	38.447	2.041	5.599	21.988	3.062	13.927	5.765	1.078	18.704
chr3.CM0228.100.r2.m	cu zu superoxide dismutase	32.960	2.064	6.261	5.058	3.640	6.440	1.449	5.764	14.448
chr5.CM0127.240.r2.m	uncharacterized protein	26.350	2.048	1.729	0.840	0.439	4.039	11.511	0.161	2.086
chr5.CM0177.360.r2.a	glutamine expressed	25.349	1.116	4.404	8.071	0.308	3.819	8.525	0.199	2.336
chr1.CM0104.2710.2.r2.m	uncharacterized protein loc10078840 isoform 1	23.502	1.019	4.334	7.393	0.162	2.187	7.912	0.155	1.963
chr5.CM0048.402.r2.d	uncharacterized protein	21.115	1.018	0.082	15.937	0.387	4.487	8.890	0.158	2.080
chr4.CM0048.160.r2.m	aspartate N-peptidase	21.090	1.969	9.938	13.889	1.750	12.785	8.840	0.984	11.128
chr5.CM0017.1450.2.r2.m	farneylated protein	20.628	1.855	8.995	8.995	0.001	9.491	8.621	0.024	10.021
chr5.CM0006.210.r2.m	dehydration-induced protein ERD15	20.060	0.939	4.577	4.577	0.079	12.255	9.966	0.040	1.015
chr4.CM0006.210.r2.m	dehydration-induced protein ERD16	19.692	1.156	0.087	9.661	0.011	0.110	9.247	0.183	2.218
chr3.CM0590.380.r2.d	uncharacterized protein	18.423	1.475	8.007	8.501	0.456	5.367	7.538	0.745	9.981
chr2.CM0060.140.r2.m	bifunctional inhibitor lipid-transfer protein storage 2S albumin-like protein	17.858	0.442	2.474	3.498	0.209	8.866	6.702	0.539	8.038
chr5.CM0123.160.r2.d	uncharacterized protein	17.145	0.275	2.474	2.474	0.000	3.019	3.170	0.000	2.040
chr1.CM0103.220.r2.m	serine threonine kinase	17.116	0.933	5.523	5.523	0.000	1.877	1.877	0.000	2.114
chr5.CM0123.110.r2.m	desmoglein protein	16.965	0.542	5.523	5.523	0.000	3.353	3.461	0.000	2.445
LsGA_019223.1	anthranilate N-hydroxycinnamoyl benzoyltransferase	11.147	0.312	2.802	5.775	0.097	1.884	3.788	5.379	5.379
chr4.CM0026.1170.r2.d	uncharacterized protein	10.873	0.901	8.285	8.944	0.417	4.667	5.347	0.469	8.774
chr5.CM0026.1170.r2.d	uncharacterized protein	10.849	0.422	3.935	3.935	0.001	4.621	3.747	0.377	3.747
LsGA_023879.1	bifunctional inhibitor lipid-transfer protein storage 2S albumin-like protein	10.812	0.886	8.191	2.735	0.184	6.718	5.152	0.368	7.135
chr3.CM0036.100.r2.m	uncharacterized protein atg2275-like	10.784	0.703	6.518	2.709	0.037	1.374	3.748	0.272	7.265
chr5.CM0036.100.r2.m	polygalacturonase atg4100-like	10.367	0.128	1.238	1.238	0.000	1.044	3.925	0.169	4.114
chr5.CM0006.200.r2.m	uncharacterized protein	10.309	0.618	9.585	1.030	0.131	12.720	1.124	0.200	18.309
chr5.CM0006.200.r2.m	lipoxygenase	10.290	0.321	1.237	1.237	0.000	1.030	3.925	0.169	3.925
chr5.CM0237.110.r2.m	lipoxygenase	1								

LsGSA_0436921.1	expandin B1	1.640	0.126	7.695	1.382	0.072	5.193	0.452	0.038	8.351
LsGSA_0436921.1	probable phosphotransferase	1.626	0.033	6.346	0.569	0.020	8.523	0.672	0.045	6.683
LsGSA_0436921.1	probable phosphotransferase-like	1.600	0.037	5.420	0.511	0.020	8.544	0.490	0.033	6.900
LsGSA_0993481.1	hexose transporter	1.595	0.099	6.226	0.596	0.025	8.839	0.731	0.062	8.463
LsGSA_0186932.1	struB-like/receptor family 6	1.590	0.084	5.309	0.563	0.021	3.775	0.707	0.045	6.422
LsGSA_0436921.1	arginine/serine-rich domain	1.586	0.000	5.001	0.491	0.020	6.437	0.584	0.025	4.222
LsGSA_0194981.1	uncharacterized protein	1.559	0.066	4.223	0.156	0.018	11.443	0.508	0.037	10.469
LsGSA_0194981.1	uncharacterized protein	1.550	0.146	9.414	0.412	0.030	7.272	0.558	0.061	10.867
chr3:CM00911.130>2.2d	glucavolase	1.544	0.130	8.430	0.451	0.025	6.426	0.887	0.057	8.361
chr3:CM00911.130>2.2d	glutathione reductase	1.486	0.034	3.668	0.145	0.025	5.140	0.176	0.020	3.31
LsGSA_0755931.1	halacid dehalogenase-like hydrolase domain-containing protein	1.482	0.049	3.341	0.668	0.070	10.434	0.614	0.022	3.649
LsGSA_148212.1	ER glycerol-phosphate acyltransferase	1.458	0.092	6.305	0.366	0.007	1.855	0.548	0.037	6.793
LsGSA_0436921.1	probable nucleic acid TESTA 1 protein	1.432	0.079	5.301	0.333	0.005	7.225	0.702	0.018	3.93
chr3:CM00911.1820>2.2m	enhanced disease susceptibility	1.432	0.113	7.859	0.183	0.047	25.630	0.280	0.035	12.640
chr4:CM00421.1810>2.2m	RING finger protein	1.426	0.100	7.028	0.257	0.010	3.959	0.628	0.086	13.616
LsGSA_0019881.1	rhodanescinuctonate lyase	1.415	0.111	7.819	0.165	0.017	14.658	0.392	0.007	1.679
LsGSA_015152.1	uncharacterized protein	1.407	0.113	8.027	0.086	0.006	1.071	0.131	0.027	1.444
LsGSA_1002231.1	uncharacterized protein	1.403	0.080	5.737	0.337	0.007	1.968	0.837	0.003	0.609
LsGSA_0249450.1	mature efflux family protein dck1-like	1.400	0.664	47.428	0.164	0.064	43.056	0.072	0.022	30.260
chr2:CM00911.1820>2.2m	protein kinase	1.393	0.079	5.536	0.242	0.022	6.742	0.450	0.021	2.00
chr8:CM00371.1460>2.2d	protein ME21-like 2	1.321	0.067	5.089	0.149	0.015	10.037	0.832	0.062	9.750
chr5:CM00521.160>2.2d	dihydroflavonol reductase	1.307	0.013	0.974	0.247	0.022	9.087	0.570	0.010	1.735
chr8:CM00211.220>2.2d	UDP-glucuronyl epimerase	1.285	0.107	8.352	0.231	0.073	0.973	0.834	0.038	9.38
chr3:CM00101.160>2.2m	uncharacterized protein	1.271	0.018	1.447	0.317	0.004	0.480	0.576	0.007	1.717
chr2:CM02491.1490>2.2m	aldose 1-epimerase family protein	1.268	0.007	0.581	0.284	0.009	3.196	0.543	0.017	3.107
LsGSA_0350931.1	o'-acyltransferase wld1-like	1.255	0.490	39.069	0.642	0.024	38.730	0.191	0.015	39.404
chr6:CM00521.160>2.2m	tryptophanyl transferase	1.230	0.029	4.761	0.242	0.011	4.444	0.395	0.010	1.14
LsGSA_062924.1	serine carboxypeptidase-like protein	1.222	0.082	6.689	0.167	0.010	6.103	0.107	0.003	7.443
chr1:CM01051860>2.2m	RNA-binding region containing	1.207	0.074	6.124	0.121	0.009	0.332	0.272	0.027	10.055
chr2:CM00911.1820>2.2m	uncharacterized protein	1.199	0.000	7.530	0.269	0.003	8.931	0.300	0.022	2.720
LsGSA_025112.1	B-type receptor-associated like protein	1.192	0.078	6.536	0.291	0.030	10.221	0.477	0.054	11.963
chr2:CM00211.2030>2.2m	aspartic proteinase nephelepin-2	1.187	0.049	4.149	0.084	0.071	8.066	0.457	0.037	8.121
chr1:CM03221.80>2.2d	uncharacterized protein	1.184	0.068	5.756	0.051	0.091	10.663	0.333	0.027	8.220
LsGSA_022021.1	uncharacterized protein	1.165	0.000	0.729	0.221	0.021	0.231	0.272	0.005	1.478
chr6:CM00131.470>2.2d	sau33 - auxin responsive saur family member	1.161	0.007	0.609	0.440	0.022	5.056	0.220	0.031	14.025
chr5:CM03261.920>2.2m	protein phosphatase 20 15	1.124	0.085	7.545	0.311	0.011	2.995	0.486	0.018	3.768
chr2:CM00101.1820>2.2m	tryptophanyl transferase	1.106	0.035	9.538	0.148	0.006	30.116	0.313	0.004	11.014
LsGSA_010711.1	willy transcription factor	1.098	0.002	0.161	0.213	0.008	3.594	0.232	0.008	3.382
chr6:CM00581.850>2.2d	2'-oxoglutarate malate translocator	1.081	0.107	9.934	0.392	0.036	9.078	0.515	0.030	5.874
LsGSA_014252.1	glucan-beta-1,6-glucosidase	1.064	0.088	0.877	0.177	0.010	5.925	0.189	0.009	4.631
chr6:CM03139.1100>2.2d	auxin-induced protein SNF4-like	1.055	0.073	6.957	0.421	0.134	31.870	0.363	0.076	21.031
chr5:CM00525.250>2.2d	uncharacterized protein	1.046	0.007	0.649	0.290	0.025	8.761	0.383	0.011	2.952
chr2:CM00141.1820>2.2d	uncharacterized protein	1.021	0.000	2.311	0.212	0.025	9.275	0.333	0.005	1.606
chr2:CM00101.470>2.2d	no hit	1.013	0.030	3.579	0.433	0.016	3.674	0.449	0.018	4.044
chr4:CM00441.310>2.2d	penicillipopeptide repeat-containing protein	0.999	0.060	5.992	0.411	0.055	13.298	0.375	0.017	4.430
chr7:CM00211.1820>2.2m	uncharacterized protein	0.989	0.070	7.126	0.263	0.024	4.981	0.477	0.037	4.962
chr5:CM00201.1570>2.2m	AMP-binding protein	0.989	0.045	4.549	0.296	0.021	7.030	0.469	0.025	5.225
chr5:CM00968.670>2.2d	no hit	0.986	0.052	5.318	0.645	0.004	0.665	0.391	0.007	1.789
chr3:CM14681.210>2.2d	2'-oxoglutarate malate reductase	0.966	0.063	6.558	0.432	0.014	3.152	0.441	0.002	0.522
chr2:CM00101.1820>2.2m	methionine sulfoxide reductase	0.950	0.005	9.926	0.220	0.003	14.760	0.486	0.001	4.432
chr1:CM00011.400>2.2d	ribosomal RNA-processing protein 8-like	0.946	0.040	4.187	0.703	0.032	4.525	0.432	0.019	4.309
chr3:CM12613.290>2.2d	disease resistance response	0.942	0.024	2.580	0.722	0.167	23.167	0.242	0.008	3.445
chr2:CM00811.1820>2.2d	serine-threonine kinase	0.919	0.011	1.160	0.121	0.001	1.241	0.237	0.013	5.617
LsGSA_022021.1	diphend oxidase laccase	0.912	0.090	8.939	0.141	0.004	3.009	0.301	0.029	9.678
chr8:CM00211.1820>2.2d	auxin response factor 8-like	0.911	0.033	3.640	0.117	0.007	6.457	0.402	0.004	0.942
chr6:CM00849.420>2.2d	tryptophanyl transferase	0.904	0.001	0.776	0.322	0.003	3.933	0.446	0.014	1.171
chr1:CM0211.210>2.2m	ornithine decarboxylase	0.899	0.026	2.863	0.223	0.019	8.698	0.441	0.016	3.643
chr1:CM02951.1200>2.2s	tyrosine decarboxylase	0.882	0.072	8.204	0.452	0.024	11.829	0.404	0.018	11.895
LsGSA_016711.1	uncharacterized protein	0.871	0.002	0.777	0.277	0.005	1.042	0.160	0.003	0.555
chr4:CM00871.1570>2.2d	tyrosine kinase	0.866	0.024	2.821	0.258	0.007	2.580	0.430	0.011	9.563
chr3:CM15741.510>2.2d	auxin-induced protein AX28-like	0.865	0.022	4.291	0.411	0.003	0.658	0.283	0.019	8.674
LsGSA_02080.1	anthroquinone N-hydroxyquinone chloroplastic-like	0.860	0.000	0.776	0.200	0.003	1.000	0.164	0.003	3.441
chr4:CM529.40>2.2d	acyl-CoA dehydrogenase-like hydrolase domain-containing protein	0.836	0.044	5.211	0.396	0.020	2.124	0.335	0.066	12.164
LsGSA_024944.1	uncharacterized protein	0.824	0.071	8.596	0.195	0.009	4.420	0.304	0.056	18.472
chr2:CM00808.870>2.2d	tryptophanyl transferase bHLH135-like	0.817	0.078	5.956	0.141	0.004	3.307	0.290	0.013	4.332
chr2:CM02491.220>2.2d	early nodulin-like protein 1-like	0.808	0.072	8.899	0.399	0.044	10.431	0.186	0.015	8.123
chr8:CM0821.190>2.2d	uncharacterized protein	0.806	0.032	3.940	0.190	0.001	1.115	0.195	0.021	10.910
chr2:CM02391.60>2.2m	uncharacterized protein	0.790	0.007	4.459	0.206	0.003	6.140	0.384	0.006	1.779
LsGSA_016251.1	junior 1 domain	0.789	0.069	8.759	0.106	0.011	10.518	0.094	0.006	6.313
chr2:CM02303.60>2.2m	uncharacterized protein	0.780	0.012	4.234	0.206	0.003	12.921	0.120	0.003	1.016
LsGSA_014931.1	phytol kinase	0.776	0.011	4.926	0.205	0.003	1.011	0.120	0.003	0.200
chr4:CM00743.190>2.2d	glucosidase surface protein chloroplastic-like	0.774	0.036	7.187	0.087	0.011	13.091	0.091	0.003	3.422
chr3:CM1833.260>2.2d	glucosidase	0.774	0.003	0.651	0.200	0.009	38.952	0.074	0.001	1.382
LsGSA_038408.1	L-type channel	0.768	0.006	5.934	0.080	0.009	10.301	0.169	0.003	7.848
LsGSA_018189.1	SW/SNF-related matrix-associated protein/actin-dependent protein of atrin subfamily A-like protein	0.765	0.001	1.466	0.200	0.003	1.035	0.112	0.003	0.92
LsGSA_176252.1	tryptophanyl transferase	0.759	0.005	5.729	0.240	0.001	2.775	0.189	0.001	11.571
chr2:CM1285.240>2.2d	uncharacterized protein	0.746	0.022	4.789	0.100	0.004	3.783	0.175	0.000	0.968
chr8:CM245.500>2.2d	zinc finger protein constans-like 5-like	0.740	0.027	8.310	0.313	0.003	1.028	0.224	0.016	6.985
chr3:CM00521.1820>2.2m	uncharacterized protein	0.737	0.027	4.745	0.254	0.002	12.672	0.264	0.035	13.238
chr2:CM00725.500>2.2d	uncharacterized protein	0.736	0.001	6.891	0.235	0.003	3.588	0.196	0.006	1.550
chr5:CM00752.500>2.2d	profilin protein A2	0.735	0.036	6.186	0.110	0.007	6.985	0.186	0.016	19.871
chr3:CM00725.300>2.2d	uncharacterized protein	0.734	0.020	4.240	0.347	0.006	5.111	0.200	0.002	0.959
chr1:LT1429.170>2.2d	cyclin-dependent kinase F21-2-like	0.730	0.021	4.965	0.233	0.003	0.598	0.136	0.018	1.487
LsGSA_021876.1	ATP binding protein	0.727	0.035	7.723	0.177	0.018	10.079	0.067	0.005	6.970
chr4:CM00746.190>2.2d	ATP binding protein	0.723	0.030	7.697	0.177	0.018	3.037	0.1		

LsGSA_030160.1	flavin-containing monooxygenase FMO GS-OX5	0.169	0.006	3.268	0.064	0.007	8.458	0.074	0.001	1.766
LsGSA_030160.1	o-regulated protease inhibitor 17 protein	0.167	0.008	4.895	0.040	0.002	6.165	0.074	0.010	13.271
LsGSA_030160.1	ribosomal protein BiP-like RNA small subunit methyltransferase B	0.165	0.000	0.121	0.024	0.003	2.231	0.069	0.005	0.507
LsGSA_050157.1	Nucleic acid binding small subunit methyltransferase	0.161	0.011	6.950	0.093	0.002	2.221	0.064	0.000	0.339
chr6:CM0041.120.-2.d	histone H3	0.161	0.004	2.681	0.033	0.001	3.855	0.051	0.003	6.181
LsGSA_045334.1	GDSL esterase/lipase CPTRD4	0.157	0.007	4.999	0.056	0.006	24.469	0.060	0.004	1.446
chr2:CM0435.840.-2.m	RNA polymerase II C-terminal domain phosphatase-like 4-like	0.156	0.005	3.030	0.108	0.024	22.968	0.073	0.006	8.382
chr1:CM0121.120.-2.m	fasciclin-like adhesion-selectin protein 11-like	0.153	0.012	8.054	0.068	0.010	15.308	0.026	0.001	2.820
LsGSA_005131.1	WEE1-like protein kinase	0.152	0.002	1.247	0.036	0.004	12.125	0.059	0.004	0.664
chr4:CM0044.520.-2.d	uncharacterized protein	0.151	0.001	0.827	0.041	0.001	2.431	0.075	0.000	10.119
chr6:CM0041.20.-2.d	uncharacterized protein	0.147	0.008	5.622	0.043	0.004	9.706	0.067	0.005	8.920
LsGSA_082920.1	transmembrane protein bHLH122	0.139	0.010	7.015	0.081	0.000	0.138	0.068	0.004	0.765
LsGSA_065093.1	male sterility 5 family protein	0.139	0.004	2.825	0.036	0.004	12.043	0.054	0.005	9.589
LsGSA_065093.1	bifunctional nuclease BFNI	0.138	0.009	6.301	0.025	0.000	35.813	0.050	0.000	0.000
LsGSA_065093.1	14-3-3 sigma protein	0.135	0.006	4.501	0.019	0.002	12.514	0.030	0.001	0.422
chr5:CM125.1050.-2.a	patatin group A-3-like	0.128	0.011	8.533	0.027	0.005	20.079	0.042	0.006	14.089
chr1:CM554.420.-2.d	GDSL esterase lipase	0.128	0.008	6.464	0.027	0.021	26.665	0.046	0.013	25.891
chr5:CM0041.120.-2.m	beta-1,4-galactosidase family protein	0.126	0.006	5.908	0.034	0.004	1.915	0.056	0.006	0.798
chr1:L101F21.121.-2.d	F-actin capping protein subunit alpha	0.111	0.003	2.977	0.051	0.006	11.418	0.045	0.007	16.512
chr5:CM2020.190.-2.d	MADS-box transcription factor	0.108	0.010	9.426	0.026	0.003	12.466	0.030	0.009	12.503
LsGSA_010022.2	probable glutathione S-transferase-like	0.107	0.004	3.317	0.029	0.012	30.019	0.031	0.001	0.001
LsGSA_010022.2	uncharacterized protein	0.106	0.000	3.335	0.036	0.003	4.734	0.022	0.001	4.170
chr4:L173M20.250.-2.d	beta-ketacyl-coa synthase family protein	0.105	0.000	7.171	0.036	0.004	14.218	0.048	0.001	2.386
LsGSA_044744.1	GDSL esterase lipase	0.104	0.004	4.278	0.035	0.003	7.745	0.041	0.007	15.990
chr2:CM0041.20.-2.d	leucine-rich repeat receptor-like protein kinase lg8400-like	0.104	0.004	3.902	0.037	0.009	16.154	0.030	0.000	0.001
chr4:CM0044.1310.-2.d	esterase-like protein repeat receptor-like protein kinase atlg8400-like	0.103	0.005	4.548	0.037	0.006	14.899	0.029	0.006	20.898
LsGSA_07008.2	GRF 1-interacting factor 3	0.102	0.007	6.775	0.021	0.001	3.684	0.028	0.001	3.997
LsGSA_06851.1	leucine-rich repeat receptor-like protein kinase lg8400-like	0.101	0.007	7.209	0.038	0.002	8.045	0.036	0.003	0.042
LsGSA_03242.80.-2.d	cytochrome P450 14-beta-hydroxylase	0.100	0.006	5.597	0.025	0.005	19.846	0.040	0.001	3.598
chr2:CM0608.1400.-2.m	protein kinase domain-containing protein	0.094	0.006	6.588	0.025	0.006	22.838	0.047	0.001	2.472
LsGSA_013282.2	probable nucleic-conjugating enzyme E2-like	0.093	0.003	3.194	0.046	0.000	10.034	0.056	0.006	17.246
chr4:CM0045.111.-2.d	uncharacterized protein	0.093	0.000	9.004	0.026	0.000	13.739	0.040	0.003	0.023
chr4:CM0045.1310.-2.d	uncharacterized protein	0.085	0.004	4.318	0.057	0.005	9.376	0.031	0.000	24.280
LsGSA_014939.1	uncharacterized protein	0.084	0.006	7.387	0.036	0.010	32.159	0.037	0.002	5.547
chr5:CM0041.120.-2.m	phosphotransferase 3-A5'-triphosphate kinase and dual-specificity protein phosphatase 2	0.074	0.003	3.933	0.018	0.002	12.305	0.030	0.000	0.000
LsGSA_028655.1	mitogen-activated protein kinase kinase kinase 1-like	0.073	0.008	8.274	0.023	0.004	15.808	0.020	0.001	5.923
LsGSA_028655.1	uncharacterized protein	0.072	0.001	1.022	0.017	0.002	8.890	0.028	0.001	2.058
chr1:CM0041.310.-2.d	N-societytransfer ESCO2-like	0.070	0.002	2.957	0.024	0.001	8.987	0.032	0.002	5.419
chr2:CM0338.230.-2.d	DUF246 domain-containing protein atlg9410-like	0.067	0.006	9.104	0.028	0.006	20.789	0.028	0.001	3.884
chr5:CM790.60.-2.d	beta-1,4-glucan phosphatase 2A 57 kDa regulatory subunit B iota isoform-like	0.064	0.006	3.425	0.026	0.006	24.521	0.030	0.001	33.599
LsGSA_039494.1	phospholipid glycan synthase family protein	0.064	0.002	3.277	0.020	0.002	5.732	0.030	0.004	1.731
chr2:CM0177.160.-2.m	cinnamyl alcohol dehydrogenase-like protein	0.060	0.001	2.339	0.027	0.003	10.188	0.029	0.007	23.167
chr4:CM0041.120.-2.m	uncharacterized protein atlg1795-like	0.059	0.004	1.869	0.039	0.002	6.125	0.027	0.003	9.306
chr4:CM0428.400.-2.m	arginine biosynthesis bifunctional protein	0.051	0.004	7.468	0.022	0.004	20.269	0.022	0.001	4.268
LsGSA_028933.2	protein methionine transferase-like 33	0.042	0.002	3.557	0.022	0.003	14.588	0.019	0.003	17.851

Supplemental Table 4. Expression profile of 42 genes in cluster 4 during nodule senescence

ID number	Annotation	2 wpi			4 wpi			8 wpi		
		Average Normalized	SD Normalized	SD Normalized	Average Normalized	SD Normalized	SD Normalized	Average Normalized	SD Normalized	SD Normalized
chr4:CM0337.590.-2.m	vacuolar iron transporter homolog 4-like	72.098	8.530	9.057	15.674	11.226	9.705	51.827	4.049	4.049
chr6:CM1514.140.-2.d	dihydroneopterin aldolase	28.401	2.476	8.718	24.968	3.690	14.778	13.890	3.401	24.485
LsGSA_030160.1	sugar transporter sweet-1-like	15.061	4.14	2.713	26.852	5.712	27.231	6.782	1.717	25.932
chr1:CM0041.120.-2.d	uncharacterized protein	13.428	2.254	16.703	20.207	5.027	22.337	8.536	1.945	20.007
chr5:L14308.110.-2.d	vegetative lectin	4.789	0.230	4.802	8.855	0.003	1.804	0.095	0.528	
chr2:CM0021.1300.-2.m	expansin-like BI-like	4.263	0.339	7.955	5.541	0.499	9.012	1.898	0.134	7.059
chr5:CM0021.120.-2.d	beta-1,4-glucan synthase-like	1.571	0.015	0.871	0.747	0.024	3.247	0.546	0.205	0.446
LsGSA_068081.2	inositol phosphate kinase	1.656	0.119	1.783	2.768	0.249	12.614	0.905	0.069	7.672
chr4:CM0037.380.-2.d	mitochondrial substrate carrier family protein	1.347	0.017	1.257	1.473	0.033	2.259	0.673	0.014	2.020
chr1:CM0041.120.-2.d	probable membrane-associated kinase regulator 4-like	1.149	0.020	1.744	1.011	0.041	3.659	0.483	0.005	1.031
chr4:CM0041.120.-2.d	probable membrane-associated kinase regulator 4-like	0.932	0.008	5.217	0.929	0.044	4.729	0.320	0.016	0.000
LsGSA_016572.1	ethylene-responsive transcription factor ERF118	0.815	0.044	5.336	0.842	0.050	5.969	0.404	0.000	0.026
chr1:L10108.110.-2.d	uncharacterized protein	0.804	0.053	6.543	1.008	0.078	7.531	0.361	0.041	11.353
LsGSA_039721.1	glucose 1-dehydrogenase-like	0.797	0.036	4.663	0.868	0.038	4.924	0.240	0.068	0.078
LsGSA_039721.1	phosphatase a2	0.751	0.023	3.104	1.040	0.039	3.788	0.314	0.020	6.309
chr2:CM0373.110.-2.d	beta-phosphatase	0.653	0.030	4.536	0.956	0.030	5.222	0.325	0.002	0.673
chr5:CM0021.1200.-2.m	expansin-BB	0.632	0.025	8.97	0.922	0.022	11.450	0.221	0.006	0.000
LsGSA_067848.1	peptide chain release factor 2	0.625	0.048	7.615	0.503	0.000	0.006	0.164	0.003	1.817
chr6:CM0021.120.-2.m	uncharacterized protein	0.609	0.008	1.307	0.693	0.023	3.375	0.238	0.007	2.779
chr5:CM0021.120.-2.d	uncharacterized protein	0.574	0.001	0.159	0.159	0.014	4.810	0.169	0.007	4.362
chr2:L144B19.150.-2.d	plasma membrane H+-ATPase	0.319	0.006	1.133	0.200	0.012	1.183	0.250	0.015	0.000
chr2:CM0788.290.-2.m	probable LRR receptor-like serine threonine-protein kinase atlg14390-like	0.490	0.000	0.081	1.609	0.032	2.020	0.196	0.000	0.080
chr5:CM0045.290.-2.d	P-2'-O-xylose 3-deoxyphosphoglycerate aldo-lactose 3'-deoxy-d-arabinohexulosonate 7-phosphate synthase	0.406	0.028	6.945	0.364	0.006	1.692	0.202	0.004	1.762
chr6:CM0055.980.-2.d	probable LRR receptor-like serine threonine-protein kinase atlg04110-like	0.380	0.022	5.761	0.311	0.018	5.864	0.190	0.026	13.509
chr5:CM0042.130.-2.m	S'-lectin protein kinase family protein	0.354	0.028	7.992	0.266	0.001	11.472	0.082	0.027	32.621
chr2:CM0041.120.-2.m	AP2 domain-containing transcription factor	0.344	0.014	3.965	0.268	0.012	2.070	0.122	0.003	1.715
chr1:CM0041.120.-2.d	uncharacterized protein	0.274	0.007	2.390	0.334	0.000	0.122	0.131	0.005	3.887
chr2:CM0081.1090.-2.m	uncharacterized protein	0.259	0.025	9.681	0.239	0.002	0.642	0.119	0.012	10.457
chr5:CM0359.140.-2.d	phytosulfokine peptide precursor	0.252	0.014	5.739	0.869	0.051	5.900	0.053	0.025	46.584
chr5:CM0042.1120.-2.m	DNA topoisomerase 6 subunit B	0.209	0.009	4.249	0.216	0.025	11.749	0.091	0.014	15.494
chr5:CM0444.290.-2.m	CDT1-like protein chloroplast-like	0.195	0.004	2.277	0.178	0.007	3.870	0.081	0.003	4.145
chr4:CM0037.540.-2.d	50S ribosomal protein D23-like	0.155	0.012	7.584	0.128	0.007	5.659	0.072	0.002	2.445
chr5:CM0205.3790.-2.m	metal ion binding protein	0.125	0.009	6.839	0.157	0.005	3.123	0.058	0.005	8.518
LsGSA_037651.1	uncharacterized protein	0.121	0.003	2.525	0.171	0.002	1.259	0.028	0.002	8.657
chr5:CM0041.120.-2.d	uncharacterized protein	0.110	0.003	2.317	0.202					

Supplemental Table 5. Primers list used in this study.

ID	Annotation	Forward primer (5'→3')	Reverse primer (5'→3')
chr1.CM0017.1030.r2.m	class I chitinase	TGTGACTTGCTTGGAGTTGG	TGTCGAGAAGGGAGCTGTGAA
chr1.CM0195.250.r2.m	cysteine proteinase 15a-like	TGGAGTTCATGCCATACA	CACCAAGAACCCATGAT
chr1.CM0544.890.r2.m	Cu/Zn superoxide dismutase	GGCCATGAGCTTAGCAAGAC	CACAAGCTACTCTGCCACCA
chr2.CM0021.3270.r2.m	osmotin precursor	GGACAGGTGCCATGATTCTT	GAAAGTGTGGTGGGATCAT
chr2.CM0610.140.r2.m	ethylene-responsive transcriptional coactivator-like protein	CGCGTTGGAGAAGAAGATG	CACCGCCTTACCATCTCAT
chr2.LjT48A12.210.r2.d	red chlorophyll catabolite reductase	TTGGTTCAAGCTTCCAAGG	ACTCCTGATGGCTTCAGT
chr3.CM0091.1820.r2.m	enhanced disease susceptibility	CTCTCACCAATGCAATT	TGCAGCTCATACTGCCCTAA
chr3.CM0116.300.r2.m	Ferritin	AAAGAGGGCATGCAGAGAAA	CCTTCACCACGAATGTCT
chr3.CM0127.770.r2.d	small heat shock protein	GACTTCCGGACAAGCTT	TGACCTTGAGCACACCATTC
chr3.CM0786.10.r2.d	zinc finger protein	CAAGGCTTGAGGGAGAAGAA	CCTCCAAGTGCTTGACCAAT
chr3.CM1570.70.r2.a	IAA-amino acid hydrolase ilr1-like protein	GCTGACTTCATGCATCATT	TAGCGATGGGTCTCTTGC
chr3.LjT36L22.160.r2.a	nicotianamine synthase	TGAAGGAGGGAGGTGTTTG	AAAGCTCTAGCCCCTTTG
chr4.CM0227.40.r2.d	AP2 domain-containing transcription factor	GGGAATGGAATTGGAAGGAG	CCCCAATCTATCCAAAT
chr4.CM0337.590.r2.m	vacuolar iron transporter homolog 4-like	GCTTTGGTGGCTTGGTAG	CTCGTCATGGGAGCTTIC
chr4.CM0414.440.r2.d	metal transporter	CATGTAGAAAAGGGACTTTG	TTCTGGTAGTGGGATACTTG
chr4.CM0432.1970.r2.m	heat shock protein	CAGTGGAAATTCCAGAGGA	AGTGAGAACCCATTCTCCA
chr4.CM0432.790.r2.m	carboxypeptidase C	CATGGAGGCCTTGGTATCAT	ATGACCAGCTCTCGAAATG
chr5.CM0096.20.r2.m	L-asparaginase	AAGCATCGTTGGATGAAGG	ATGCCGTGCAAGTAAACCC
chr6.CM0245.580.r2.a	serine acetyltransferase mitochondrial	TGGTCTAAGATTGGTGTG	TAACAACCTTGCTGGTTCC
LjSGA_022305.1	myristoyl-acyl carrier protein chloroplastic-like	TCAGAAAGACCTGACCTGCAA	GGCTCGGACAACATCTGATT
LjSGA_025506.1	BON1-associated protein 2-like	TGGTGGGTTCTTGTGGATT	CACAGAAGGGGAAACACCAAG
LjSGA_025815.1	L-ascorbate oxidase homolog	ATGTGGAATTGAGGTCTGC	GCTTTCGTGTGCATTCCATA
LjSGA_029450.1	mate efflux family protein dtx1-like	GTGGCTGGGGATCGTATGT	GGCCTCTTTCTCTTGTCT
LjSGA_031662.1	receptor-like protein 12-like	TGGTATTGGCAACTTGTGGA	CCCTTGTCTCTGTGGTTA
LjSGA_035003.1	O-acyltransferase WSD1-like	TGACTTTGTTGGCACTCCA	CCAAATCCAAGAGCATACCC
LjSGA_041970.1	OCS element-binding	AGAGCTTCGCCAAATGCTAA	CATCTTCAGAGGGGCCAAAG
LjSGA_051232.1	RING finger protein	TGGTCACACCAAACCTCTG	CTCGAGTGTTGCTATGGTCA
LjSGA_059392.1	Kunitz-type trypsin inhibitor-like 2 protein	ATGGAAGGCGTCTCATTCTG	ACACGAGCAAACACAACCTG
LjSGA_060891.2	inositol phosphate kinase	TGCTGCAGGAGTTGTGAC	GCAAGGACTTCTCTTCACG
LjSGA_085903.1	polygalacturonase	GGCAGCATATGGGGAGATAA	GGTTGCCTCTCTCAAACAA
LjSGA_130658.1	1-amino cyclopropane-1-carboxylate oxidase	AGAGTGTGGAGCATCGTGTG	GGAGCAGGGTAGATGACAGC
LjT36K06.70.r2.d	bidirectional sugar transporter sweet1-like	GCCACAAACAAAGGAGGAATC	GCTTGAGCTCCATTGCAATT
chr3.CM0996.1420.r2.m	Ubiquitin	ATGCAGATCTCGTCAAGACCTT	ACCTCCCTCAGACGAAG