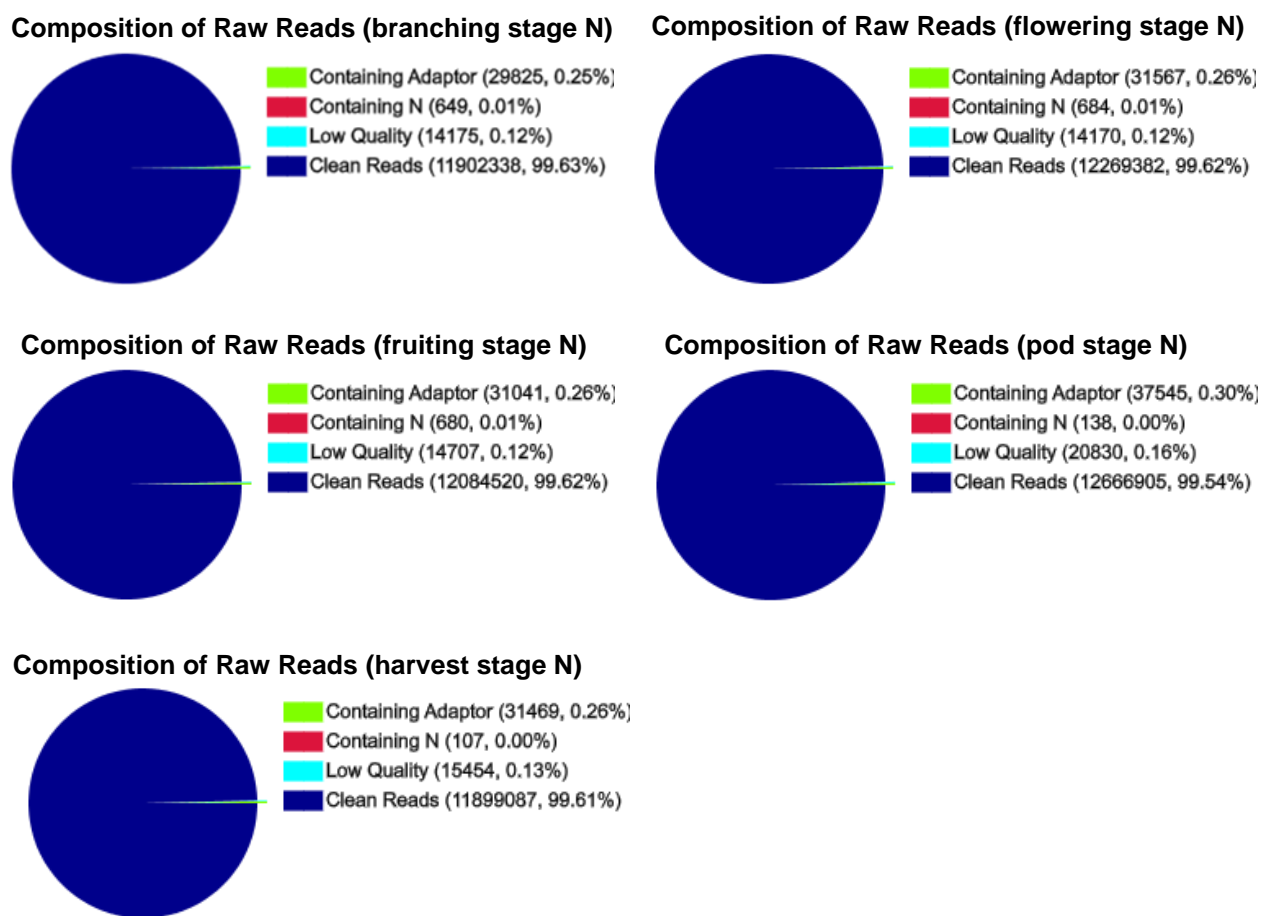


RNA-Seq analysis of nodule development at five different developmental stages of soybean (*Glycine max*) inoculated with *Bradyrhizobium japonicum* strain 113-2

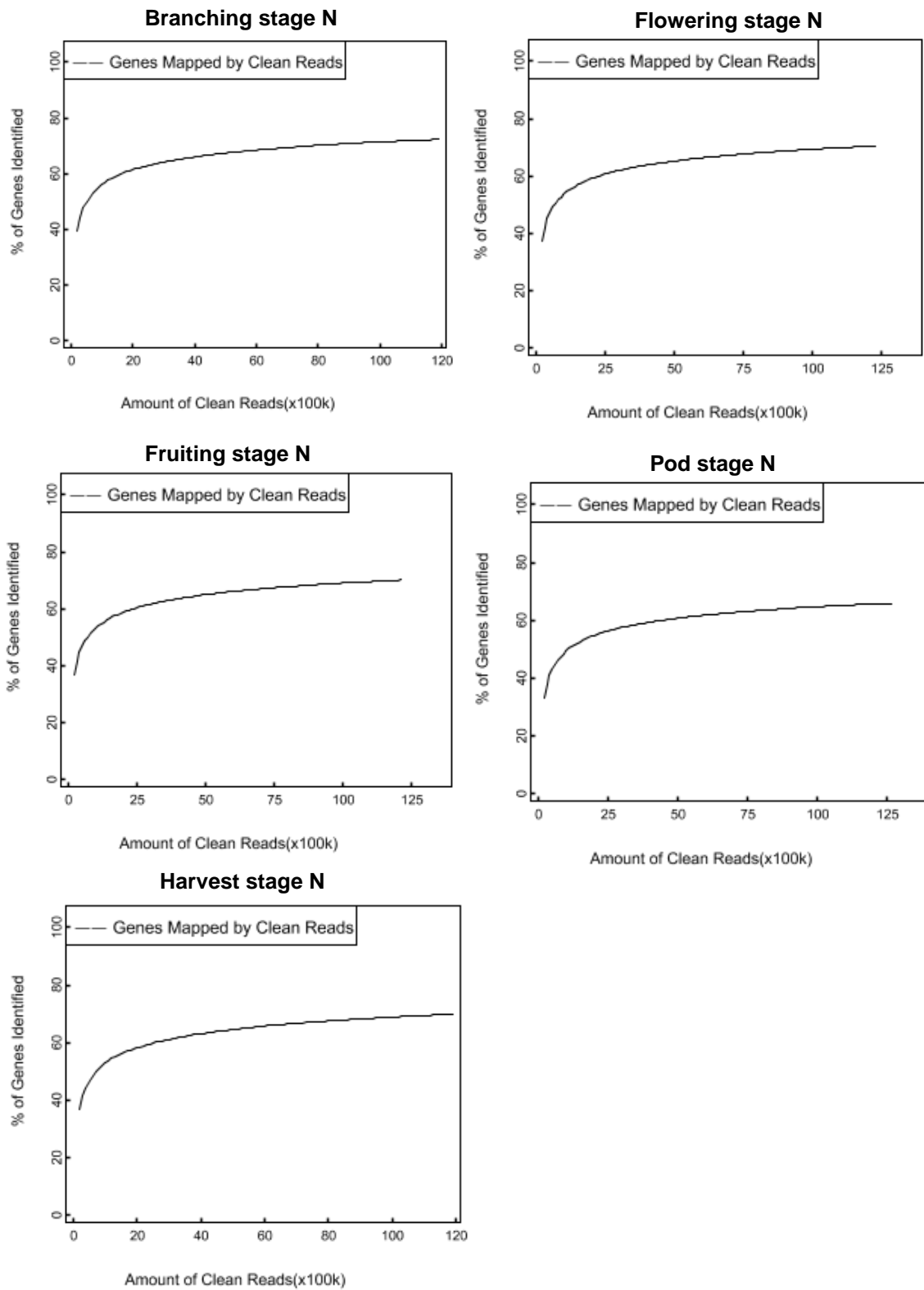
Song L Yuan<sup>1,2</sup>, Rong Li<sup>1,2</sup>, Hai F Chen<sup>1,2</sup>, Chan J Zhang<sup>1,2</sup>, Li M Chen<sup>1,2</sup>, Qing N Hao<sup>1,2</sup>,

Shui L Chen<sup>1,2</sup>, Zhi H Shan<sup>1,2</sup>, Zhong L Yang<sup>1,2</sup>, Xiao J Zhang<sup>1,2</sup>, De Z Qiu<sup>1,2</sup> and Xin A

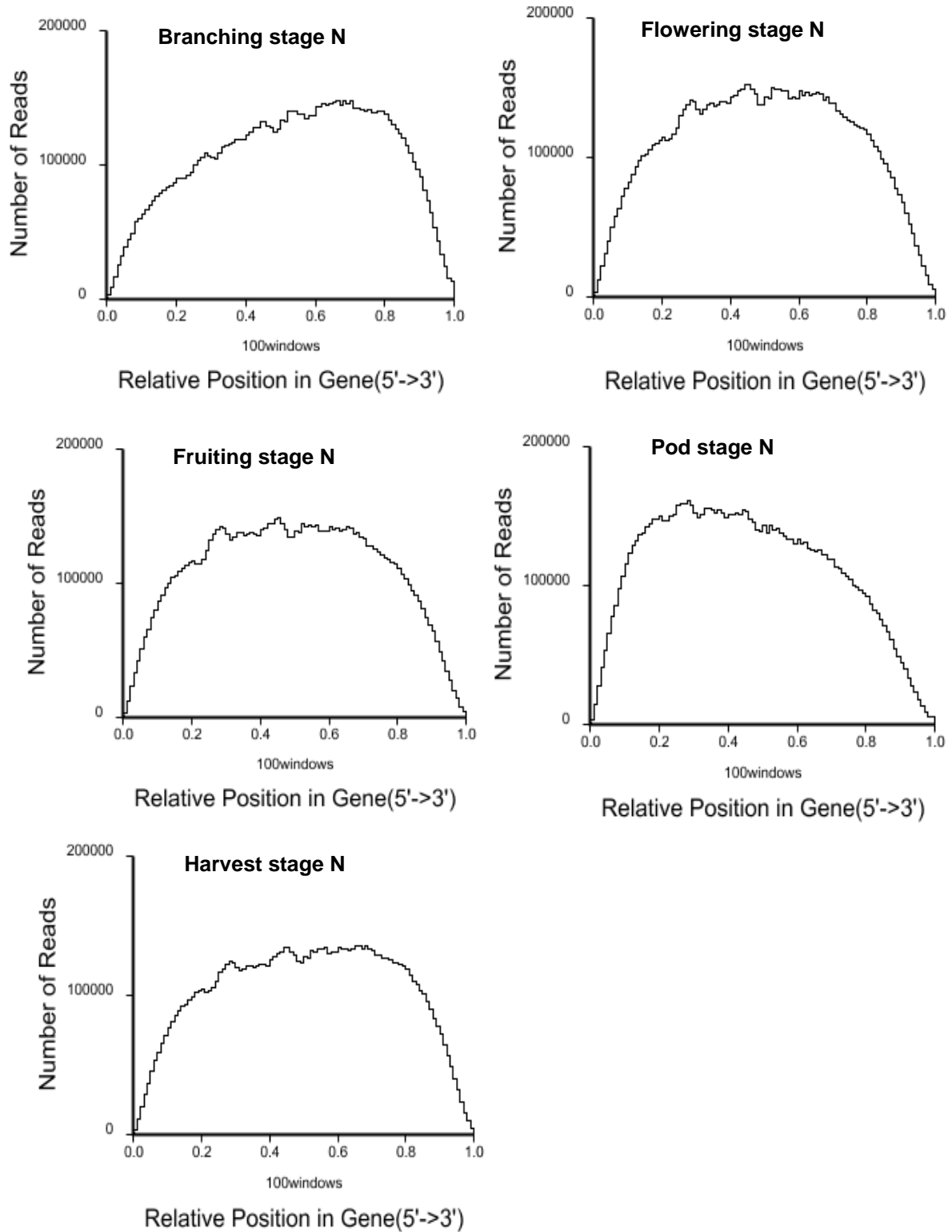
Zhou<sup>1,2\*</sup>



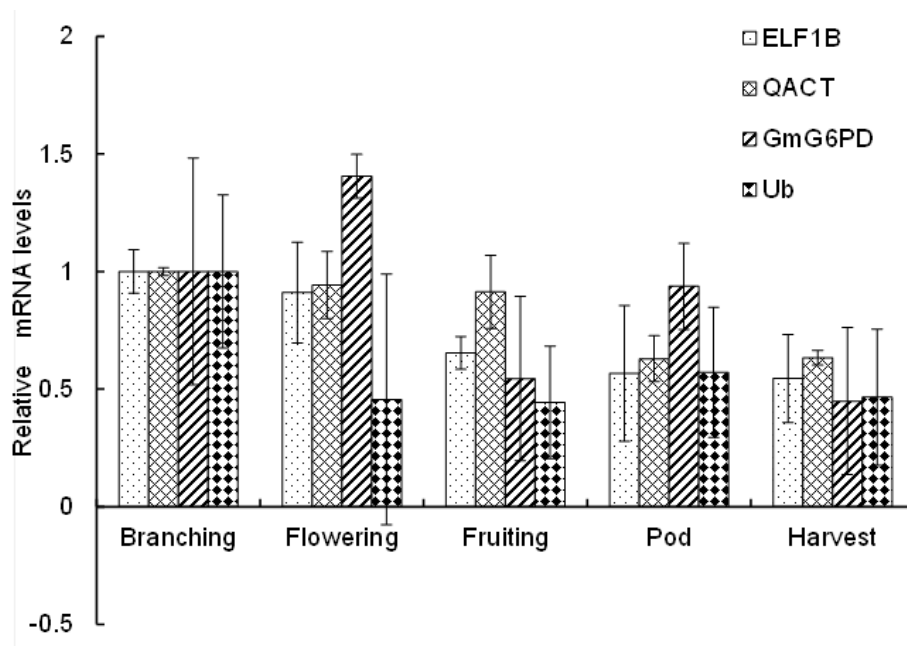
**Figure S1: Representative results of the classification of raw reads for nodules samples at five developmental periods.**



**Figure S2: Representative results of sequencing saturation analysis for nodules sampled at five developmental periods.**



**Figure S3: Representative results of distributions of reads on reference genes for nodules sampled at five developmental periods.**



**Figure S4: The stability assay of four references genes at five developmental stages of soybean.**

**Table S1: Analysis of nodulation phenotypic in soybean inoculated with rhizobium strains113-2.**

	mean value $\pm$ SE	
	Number of nodules per plant	Dry Weight per nodule(mg)
Branching	13.125 $\pm$ 1.548 (N=24)	–
Flowering	42.375 $\pm$ 1.908 (N=24)	1.366 $\pm$ 0.050
Fruiting	51.520 $\pm$ 5.337 (N=25)	2.133 $\pm$ 0.146
Pod	37.400 $\pm$ 1.830 (N=25)	1.071 $\pm$ 0.044
harvest	14.500 $\pm$ 1.558 (N=15)	1.697 $\pm$ 0.26
P Value (Branching/Flowering)	0.445987055	–
P Value (Branching/Fruiting)	0.921888575	–
P Value (Branching/Pod)	0.671463359	–
P Value (Branching/harvest)	–	–
P Value (Flowering/Fruiting)	0.136749853	–
P Value (Flowering/Pod)	0.103619795	–
P Value (Flowering/harvest)	–	–
P Value (Fruiting/Pod)	0.014539473	–
P Value (Fruiting/harvest)	–	–
P Value (Pod/harvest)	–	–

**Table S2: Summary of the mapping result (mapping to reference genes and the genome).**

	branching stage N	flowering stage N	fruiting stage N	pod stage N	harvest stage N
Map to Gene					
total mapped reads	10362371(87.06%)	10945168(89.21%)	10750603(88.96%)	11050021(87.24%)	10163957(85.42%)
perfect match reads	8894432(74.73%)	9371580(76.38%)	9140987(75.64%)	9296356(73.39%)	8646303(72.66%)
unique match reads	8881615(74.62%)	9424020(76.81%)	9268099(76.69%)	9673766(76.37%)	8811312(74.05%)
multi-position match	1480756(12.44%)	1521148(12.4%)	1482504(12.27%)	1376255(10.86%)	1352645(11.37%)
Map to Genome					
total mapped reads	10183173(85.56%)	10490977(85.51%)	10287336(85.13%)	10635747(83.96%)	9718196(81.67%)
perfect match reads	8531889(71.68%)	8736557(71.21%)	8498873(70.33%)	8706798(68.74%)	8031614(67.5%)
unique match reads	8644266(72.63%)	8991605(73.28%)	8856144(73.29%)	9300692(73.43%)	8417920(70.74%)
multi-position match	1538907(12.93%)	1499372(12.22%)	1431192(11.84%)	1335055(10.54%)	1300276(10.93%)

**Table S4: Lists of DEGs with over 8-fold changes in the plant–pathogen interaction KEGG pathway.**

Gene sets	Gene	Groups
<b>MKK1/2</b>	Glyma15g18860	Group 1,4
<b>PR1</b>	Glyma17g07350	Group 1
	Glyma13g32560	Group 1
	Glyma15g06830	Group 5,8
<b>CEBiP</b>	Glyma06g11820	Group 2,4
<b>RPS5</b>	Glyma15g02870	Group 3
	Glyma18g14810	Group 3
	Glyma08g16041	Group 3,6
	Glyma19g07680	Group 3
	Glyma18g51546	Group 6,8,10
	Glyma16g34030	Group 6,8,10
	Glyma15g39531	Group 6,8
	Glyma08g12560	Group 10
<b>PBS1</b>	Glyma06g46971	Group 5
<b>WRKY2209</b>	Glyma19g40470	Group 7,9
	Glyma16g03570	Group 7,9

<b>RIN4</b>	Glyma09g01151	Group 7,10
<b>EFR</b>	Glyma07g17910	Group 10
<b>RPS2</b>	Glyma18g51546	Group 10
	Glyma16g34030	Group 10
	Glyma08g12560	Group 10
	Glyma02g04750	Group 10
	Glyma17g21200	Group 10

**Table S5: Cysteine proteinases, cystatins and cysteine-rich proteins identified in nodule development and senescence using RNA-Seq.**

cysteine-rich genes					
Gene	Groups	Annotation	Gene	Groups	Annotation
Glyma10g35100	Group 3-4,6-9	cysteine proteinase	Glyma09g40681	Group 1-4,6,8	protein kinases
Glyma12g04340	Group 7,9	cysteine proteinase	Glyma10g39910	Group 4,10	protein kinases
Glyma17g18440	Group 1-3	cysteine proteinase	Glyma11g32301	Group 3,7,8,10	protein kinases
Glyma11g12130	Group 2-4,7	cysteine proteinase	Glyma11g32500	Group 3,6,8,10	protein kinases
Glyma14g09440	Group 3-4,6,8	cysteine proteinase	Glyma13g25800	Group 3,6,8,10	protein kinases
Glyma17g35720	Group 3-4,6-9	cysteine proteinase	Glyma09g27782	Group 1,2,4,6,8,10	protein kinases
Glyma04g03020	Group 3,10	cysteine proteinase	Glyma20g27780	Group 1,2,4,6,8,10	protein kinases
Glyma15g19580	Group 3,6	cysteine proteinase	Glyma08g39150	Group 4	protein kinases
Glyma14g40670	Group 1-7,10	cysteine proteinase	Glyma07g24010	Group 1,2,4,10	protein kinases
Glyma09g08100	Group 3-4,6-9	cysteine proteinase	Glyma20g27460	Group 1,2,4	protein kinases
Glyma04g36470	Group 1-4,6-9	cysteine proteinase	Glyma18g20470	Group 1,2,4	protein kinases
Glyma06g18390	Group 1,3-9	cysteine proteinase	Glyma20g27720	Group 2	protein kinases
Glyma18g12240	Group 1-2,4,6,8,10	cystatin	Glyma08g10030	Group 3	protein kinases
Glyma05g28250	Group 1-2,4	cystatin	Glyma13g25811	Group 1-4	protein kinases
Glyma15g36180	Group 3	cystatin	Glyma02g04210	Group 1,2,4,8,10	protein kinases
Glyma15g12211	Group 2-4,6	cystatin	Glyma11g32520	Group 3-7,9	protein kinases
Glyma14g04250	Group 1-5,7,10	cystatin	Glyma20g27580	Group 4,7	protein kinases
Glyma18g00690	Group 1-5	cystatin	Glyma18g53186	Group 1-5,7,9,10	protein kinases
Glyma10g26900	Group 1-5,7-10	secretory protein	Glyma18g45175	Group 1-4	protein kinases
Glyma13g01250	Group 1-4,7,9,10	secretory protein	Glyma04g15456	Group 1-4	protein kinases
Glyma05g34990	Group 1-5,7-8	secretory protein	Glyma11g31995	Group 2-7,9,10	protein kinases
Glyma18g04610	Group 1-4,6-7,9-10	secretory protein	Glyma20g27660	Group 1-4,7,9,10	protein kinases
Glyma08g04730	Group 2,4,5,8,10	secretory protein	Glyma20g27670	Group 1-4,7,9,10	protein kinases
Glyma20g34230	Group 2,5	secretory protein	Glyma20g27690	Group 1-4,7,9,10	protein kinases
Glyma19g35130	Group 2-5,7	secretory protein	Glyma20g27790	Group 1-4	protein kinases
Glyma14g39440	Group 3,4,7,9,10	secretory protein	Glyma11g32600	Group 2-10	protein kinases
Glyma13g38170	Group 2-7,9	secretory protein	Glyma01g45160	Group 3-4,6-10	protein kinases
Glyma14g14750	Group 2-8,10	polycomb protein	Glyma11g32041	Group 2-4,6-10	protein kinases
Glyma17g31399	Group 3,10	polycomb protein	Glyma10g39961	Group 1,3-10	protein kinases
Glyma10g39870	Group 1-4,7,9,10	protein kinases	Glyma18g05260	Group 1-9	protein kinases

**Table S6: Symbiotic nitrogen fixation - related genes identified in nodule development and senescence using RNA-Seq.**

Symbiotic nitrogen fixation - related genes			
Gene	Groups	Annotation	Potential symbiotic function
Glyma01g03470	Group 1-5,7,8,10	<i>Enod 40</i>	Nodule development
Glyma10g23790	Group 3,6,8,10	<i>Nodulin 35</i>	Assimilation of the fixed nitrogen
Glyma16g04750	Group 1-4,7,9,10	<i>ApyraseGS52</i>	Nodulation
Glyma02g06680	Group 10	<i>Calmodulin-like</i>	Nodulation
Glyma19g45310	Group 3,8,10	<i>MtDMI1-like</i>	Nodulation
Glyma09g33510	Group 3,6,8,10	<i>MtDMI2-like</i>	Nodulation
Glyma16g04410	Group 1-8,10	<i>MtERN1-like</i>	Nodulation
Glyma19g29000	Group 1-4,6,7	<i>MtERN1-like</i>	Nodulation
Glyma01g35255	Group 2-4,6,8,10	<i>MtIPD3-like</i>	Nodulation
Glyma09g34695	Group 3-4,6-8,10	<i>MtIPD3-like</i>	Nodulation
Glyma10g33851	Group 6,8,10	<i>MtLIN-like</i>	Nodulation
Glyma02g43860	Group 1-9	<i>MtLYK3-like</i>	Nodulation
Glyma02g43850	Group 1-6,9,10	<i>MtLYK3-like</i>	Nodulation
Glyma01g38550	Group 1-3,7,9-10	<i>MtLYR3-like</i>	Nitrate transporter
Glyma04g00210	Group 3-4	<i>MtNIN-like</i>	Nodulation
Glyma07g04430	Group 4,7,9	<i>MtNSP1-like</i>	Nodulation
Glyma06g11610	Group 2,4,5,7-9	<i>MtNSP2-like</i>	Nodulation
Glyma10g07190	Group 1-8,10	<i>MtRRP1-like</i>	Nodulation
Glyma03g33850	Group 7	<i>MtSKL1-like</i>	Nodulation
Glyma12g04390	Group 1-6,10	<i>MtSUNN-like</i>	Regulates nodule number
Glyma20g38950	Group 3,6,8	<i>GmN56</i>	Nitrogen fixation
Glyma13g12484	Group 6	<i>GmN56</i>	Nitrogen fixation
Glyma19g29880	Group 1-7,9,10	<i>GmN56</i>	Nitrogen fixation
Glyma05g08400	Group 1-4,7-9	<i>GmENOD93</i>	Nodule development
Glyma17g12610	Group 1,2,6,8,9	<i>GmENOD93</i>	Nodule development

**Table S7: Primers for genes selected for semi-quantitative RT-PCR and qPCR**

**analysis**

Gene	Forward primer	Reverse primer
<i>Ubiquitin</i> (soybean)	5'-GCTCGTTGTGTAATGTTGG-3'	5'-CGTAGGTGGGATATTAGAGT-3'
<i>QACT</i> (soybean)	5'-ATCTTGACTGAGCGTGGTTATTCC-3'	5'-GCTGGTCCTGGCTGTCTCC-3'
<i>ELF1B</i> (soybean)	5'-GTTGAAAAGCCAGGGGACA-3'	5'-TCTTACCCCTTGAGCGTGG-3'

<i>Gmg6po</i> (soybean)	5'-ACTCCTTGATACCGTTGTCCAT-3'	5'-GTTTGTATCCGCCTACAGCCT-3'
<i>Glyma06g11730</i>	5'-GTCACGTGTGAACTACCAGGA-3'	5'-AGCTATATGGCCTAATCTCTGGA-3'
<i>Glyma20g34140</i>	5'-ACGGCTTGATAAGGCTCCAAA-3'	5'-AGGGTTACACAATAACTCGCC -3'
<i>Glyma06g18390</i>	5'-TCTCAGATTCAATGCCAGTGT-3'	5'-TGAAACTCTTGCCATAGAAAGG-3'
<i>Glyma05g26800</i>	5'-TGTGAATCCCCCTTCTTCAGC-3'	5'-GACAACACAAGTTCTGCAGTT-3'
<i>Glyma14g07870</i>	5'-GCCTCACTGGTATGTGACTGT-3'	5'-TGGACGTGAACAAAGAAGAGGT-3'
<i>Glyma15g13290</i>	5'-TTATTCACGCAAGCTGGGTTC-3'	5'-GGAGGTCAAATAAACAAAGGAGC-3'
<i>Glyma03g29670</i>	5'-ACTGTGGCATGTGGAGGAAC-3'	5'-TGTCAAACCACTTTTCATGGCTC-3'
<i>Glyma08g06690</i>	5'-TGGTCCTTTTCTTTCAGCACCT-3'	5'-CAGCCAAAATAGCGAGGGA-3'
<i>Glyma10g34280 (Lb)</i>	5'-ACAATAAAGGAAGCTGTTGGCGG-3'	5'-TTACACTTTACGGCAATGCAG-3'
<i>NIN (L.japonicus)</i>	5'-AACTCACTGGAAACAGGTGCTTTC-3'	5'-CTATTGCGGAATGTATTAGCTAGA-3'
<i>Enod40 (L.japonicus)</i>	5'-CAAACTCGTTATGTTGCGG-3'	5'-CACCTC AAAGGA AGAAGA ACA-3'
<i>Lb (L.japonicus)</i>	5'-CTCCAAGCCCATGCTGAAAA-3'	5'-TGGCATCTGCAAGTGTCACTTC-3'
<i>Ubiquitin (L.japonicus)</i>	5'-TTCACCTTGCTCCGTCTTC-3'	5'-AACCAACCAGCACACACAGACAATC-3'
<i>Glyma18g12240</i>	5'-GGTACCATGGCAATGATAGGAGGC -3'	5'-GGATCCTTAGCTGGGTGCAGCATAAG - 3'

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