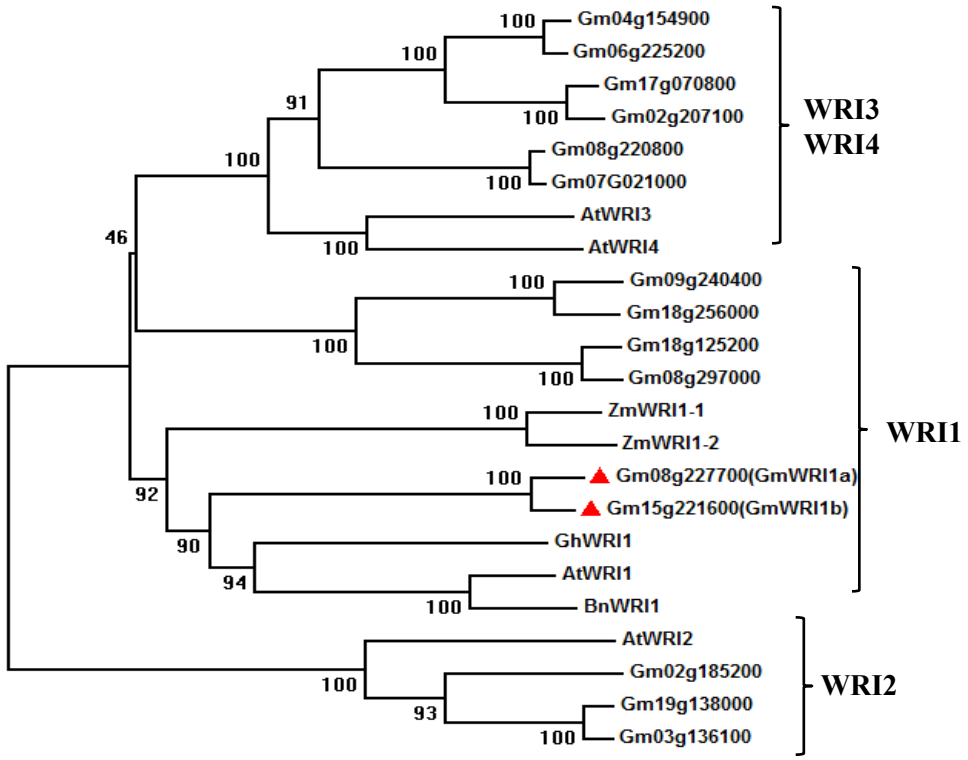
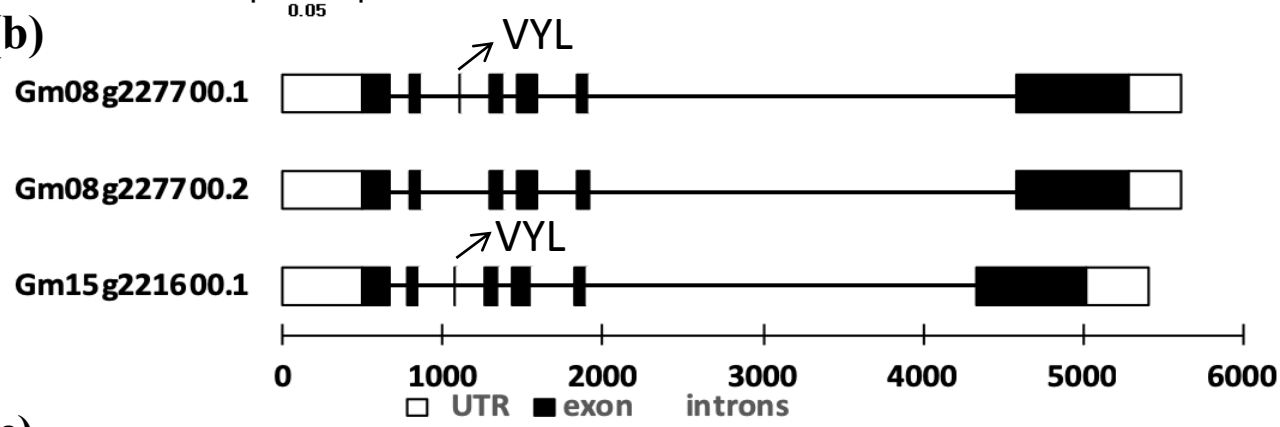
Figure S1

(a)



(b)



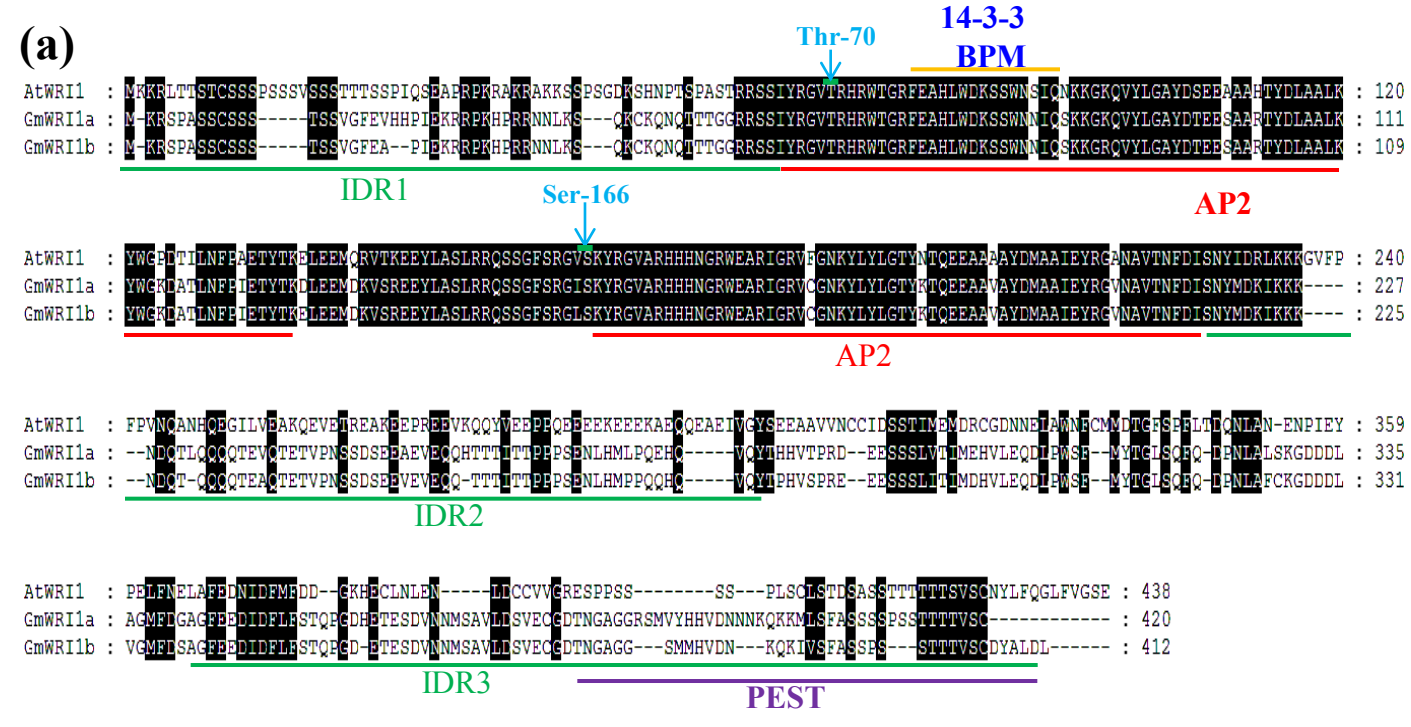
(c)

```
Gm08g.1 : MKRSPASSCSSSTSSVGFEVHHPIEKRRPKHPRRNNLKSQKCKQNQTTTGGRRSSIYRGVTRHRWTGRFEAHLWDKSSWNNIQSKKGKQVYLGAYDTEESAARTYDL : 107
Gm08g.2 : MKRSPASSCSSSTSSVGFEVHHPIEKRRPKHPRRNNLKSQKCKQNQTTTGGRRSSIYRGVTRHRWTGRFEAHLWDKSSWNNIQSKKGKQ---GAYDTEESAARTYDL : 104
Gm15g.1 : MKRSPASSCSSSTSSVGFEA--PIEKRRPKHPRRNNLKSQKCKQNQTTTGGRRSSIYRGVTRHRWTGRFEAHLWDKSSWNNIQSKKGRQVYLGAYDTEESAARTYDL : 105

Gm08g.1 : AALKYWGKDATLNFPIETYTKDLEEMDKVSREEYLASLRRQSSGFSRGISKYRGVARHHHNGRWEARIGRVCGNKYLYLGTYKTQEEAAVAYDMAAIEYRGVNAVTN : 214
Gm08g.2 : AALKYWGKDATLNFPIETYTKDLEEMDKVSREEYLASLRRQSSGFSRGISKYRGVARHHHNGRWEARIGRVCGNKYLYLGTYKTQEEAAVAYDMAAIEYRGVNAVTN : 211
Gm15g.1 : AALKYWGKDATLNFPIETYTKELEEMDKVSREEYLASLRRQSSGFSRGLSKYRGVARHHHNGRWEARIGRVCGNKYLYLGTYKTQEEAAVAYDMAAIEYRGVNAVTN : 212

Gm08g.1 : FDISNYMDKIKKKNDQTLQQQQTEVQTETVPNSSDSEEAEVEQQHTTTITTPPPSENLHMIPQEHQVQYTPHVTPRLEESSSLVTIMDHVLEQDLPWSFMYTGLSQF : 321
Gm08g.2 : FDISNYMDKIKKKNDQTLQQQQTEVQTETVPNSSDSEEAEVEQQHTTTITTPPPSENLHMIPQEHQVQYTPHVTPRLEESSSLVTIMDHVLEQDLPWSFMYTGLSQF : 318
Gm15g.1 : FDISNYMDKIKKKNDQT-QQQQTEAQTETVPNSSDSEEVEVEQQ-TTTITTPPPSENLHMPPQQHQVQYTPHVSPRLEESSSLITIMDHVLEQDLPWSFMYTGLSQF : 317

Gm08g.1 : QDPNLALSKGDDDIAGMFLQAGFEEDIDFLFSTQPGDHETESDVNNMSAVLDSVECGDTNGAGGRSMVYHHVDNNNKQKKMLSFASSSSPSSTTTTVSCDYALDL : 426
Gm08g.2 : QDPNLALSKGDDDIAGMFLQAGFEEDIDFLFSTQPGDHETESDVNNMSAVLDSVECGDTNGAGGRSMVYHHVDNNNKQKKMLSFASSSSPSSTTTTVSCDYALDL : 423
Gm15g.1 : QDPNLAFQKGDDDIVGMFLSAGFEEDIDFLFSTQPGD-ETESDVNNMSAVLDSVECGDTNGAGG---SMMHVDN---KQKIVSFASSP---SSTTTVSCDYALDL : 412
```

Figure S1. Phylogenetic (a) and gene structure analysis (b) and protein sequence alignment (c) of GmWRI1s.

(a) Unrooted phylogenetic tree of WRINKLED1s (WRI1) from various plants. The alignment was generated using Clustal and the unrooted phylogram was constructed with MEGA6 software using the neighbor-joining method,with bootstrap values based on 1000 replicates.

Figure S2



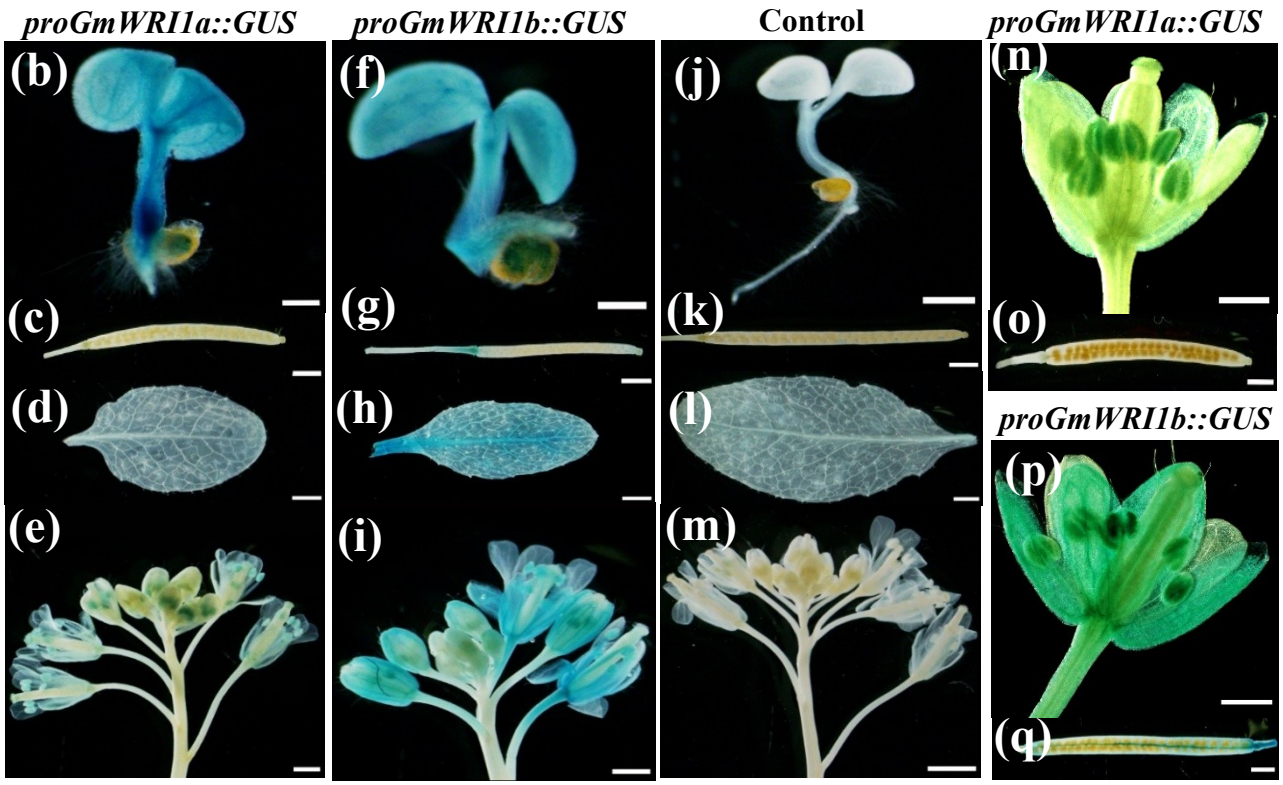


Figure S2. Schematic diagram of the GmWRI1s and AtWRI1 predicted protein sequence illustrating the locations of known domains.

(**a**) The two AP2 DNA binding domains are shown in red; three intrinsically disordered regions (IDR) are shown in green line. Two KIN10 phosphorylation sites are shown as blue bars. The14-3-3 phosphopeptide binding site and BPM binding site colocalize to the same region represented by a orange line. The C-terminal PEST domain, residues with in IDR3, is represented as a purple line.

(b-e, o,p) Distribution of GUS activities in *proGmWRI1a::GUS* Arabidopsis plants.

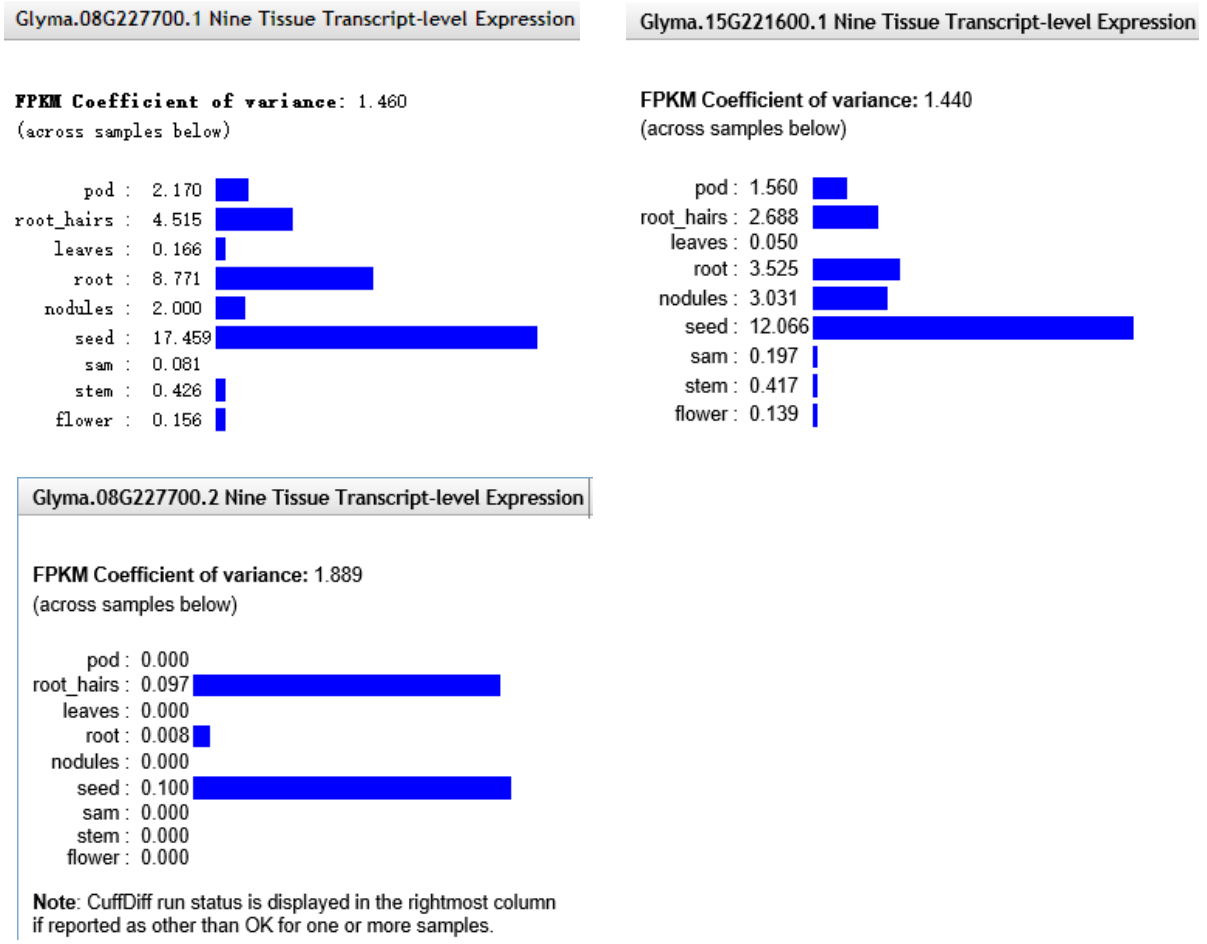
(**f-i, p,q**) Distribution of GUS activities in *proGmWRI1a::GUS* Arabidopsis plants.

(**j-m**) Distribution of GUS activities in wild-type control plants.

Histochemical detection of GUS activity in different tissues, including seedling **(b, f, j),** young pod **(c, g, k)**, leaf **(d, h, l),** inflorescences **(e, i, m),** flower (**n, p**), and old pod (**o, q**). Bars=500μm in **(b)**, (**f**), **(j)** and **(e, i, m)**, 1mm in pod , leaf and 50μm in flower.

Figure S3

(a)



(b)

From resource file Table_S5.csv (download resource file)

Raw Data: digital gene expression counts of the uniquely mappable reads.

Download List	young_leaf	flower	one cm pod	pod shell 10DAF	pod shell 14DAF	seed 10DAF	seed 14DAF	seed 21DAF	seed 25DAF	seed 28DAF	seed 35DAF	seed 42DAF	root	nodule
Glyma08g24420	3	0	1	2	0	5	34	44	112	63	133	37	24	6
Glyma15g34770	11	1	1	1	0	14	42	26	108	60	125	27	30	21

Figure S3. Public data for expression of soybean GmWRI1s

(a) Expression patterns of GmWRI1 in different tissues of soybean plant . The public date are shown in phytozme (https://phytozome.jgi.doe.gov/). *GmWRI1a* (Glyma08g227700.1 in v10, Glyma08g24420 in v9), *GmWRI1a'*(Glyma08g227700.2 in v10), *GmWRI1b* (Glyma15g 221600.1 in v10, Glyma15g34770 in v9). **(b)** *GmWRI1* mainly expressed in developing seeds. These data are retrieved from soyKB（www. SoyKB.com)

Figure S4

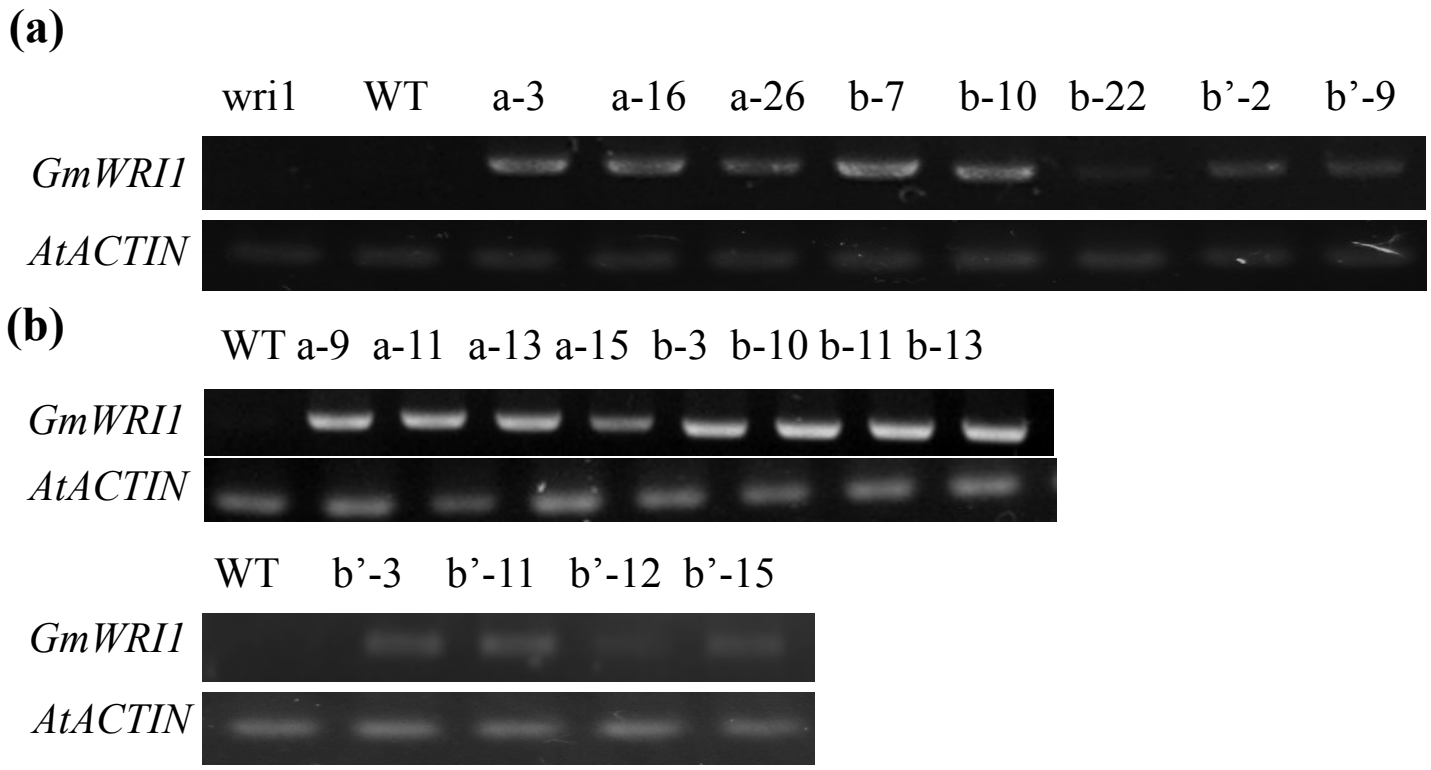


Figure S4. Confirmation of transgenic *atwri1–1* (a) and wild-type (b) plants over-expressing *GmWRI1*s.

Figure S5

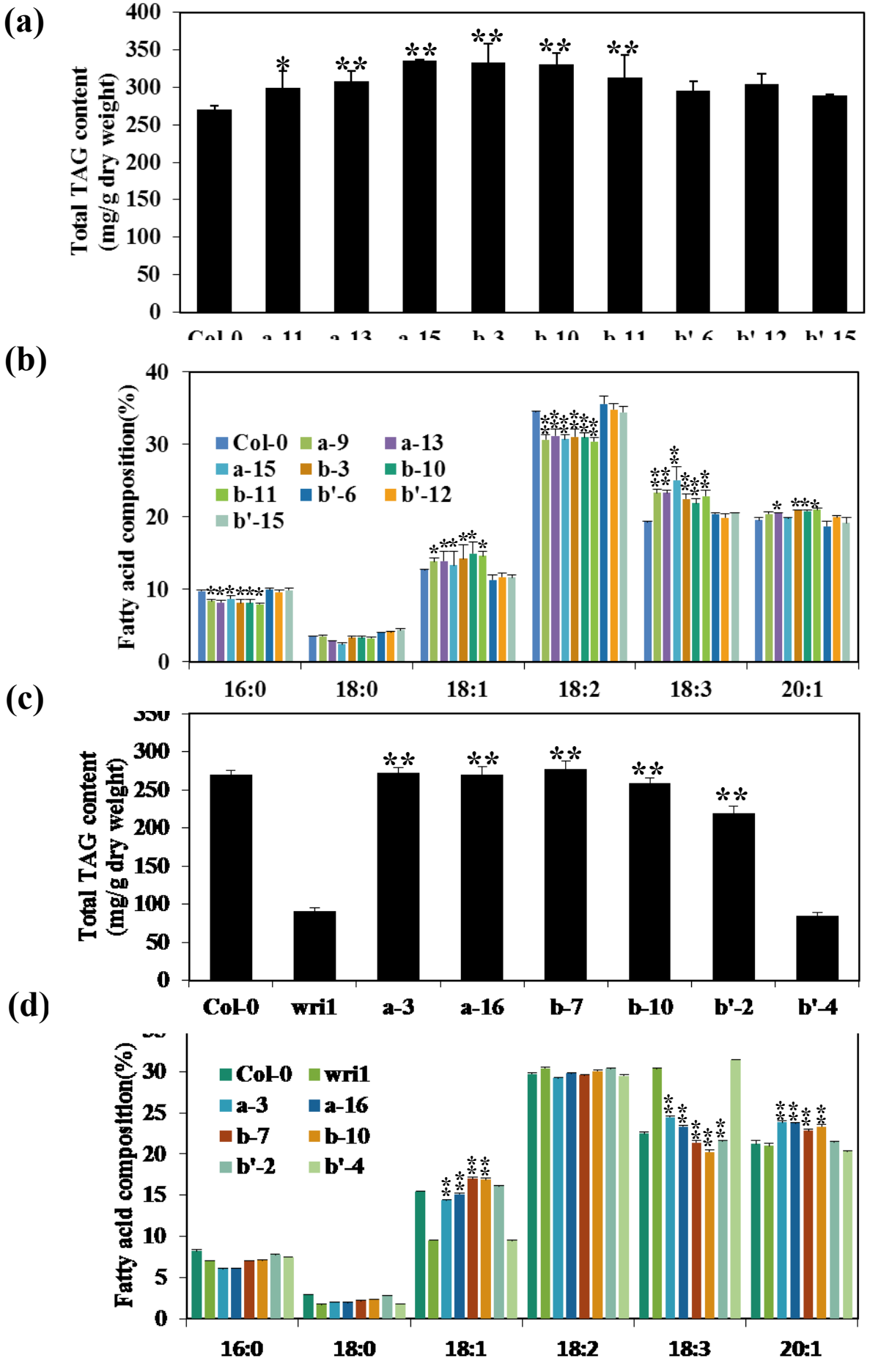

(a)
Total TAG content (mg/g dry weight)
400
350
300
250
200
150
100
50
0
Col-0
a-11
a-13
a-15
b-3
b-10
b-11
b'-6
b'-12
b'-15
(b)
Fatty acid composition(%)
40
30
20
10
0
Col-0
a-9
a-13
a-15
b-3
b-10
b-11
b'-6
b'-12
b'-15
16:0
18:0
18:1
18:2
18:3
20:1
(c)
Total TAG content (mg/g dry weight)
300
250
200
150
100
50
0
Col-0
wri1
a-3
a-16
b-7
b-10
b'-2
b'-4
(d)
Fatty acid composition(%)
30
25
20
15
10
5
0
Col-0
wri1
a-3
a-16
b-7
b-10
b'-2
b'-4
16:0
18:0
18:1
18:2
18:3
20:1

Figure S5. Functional expression of *GmWRI1a and b* in Arabidopsis thaliana seeds

(a) Total TAG contents in *GmWRI1s* expressing Arabidopsis seeds.
(b) Fatty acid composition in TAGs from wild-type Arabidopsis seeds expressing *GmWRI1s*
(c) Total TAG contents in *GmWRI1s*-complemented Arabidopsis *wri1-1* mutant seeds
(d) Fatty acid composition in TAGs from Arabidopsis *atwri1-1* mutant seeds expressing *GmWRI1s*

All data are expressed as mean ± SD from at least three biological duplicates. * $P < 0.05$ and ** $P < 0.01$ by Student's *t* test for significant difference. T3 transgenic Arabidopsis seed were used for analysis.

Figure S6

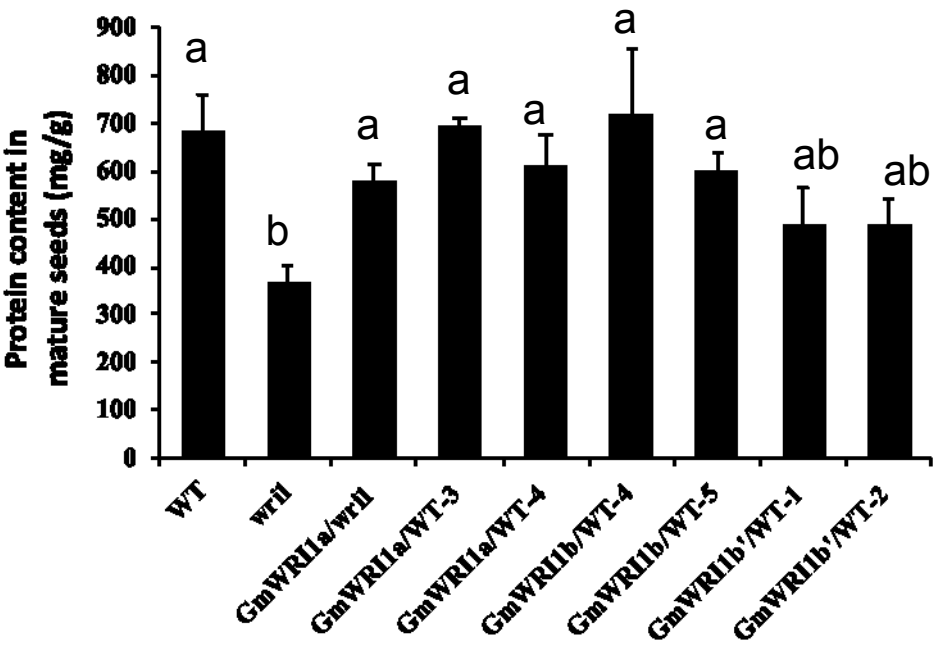


Figure S6. Proteins content of wild type, *atwri1* mutant and transgenic plant in mature seeds.

Figure S7

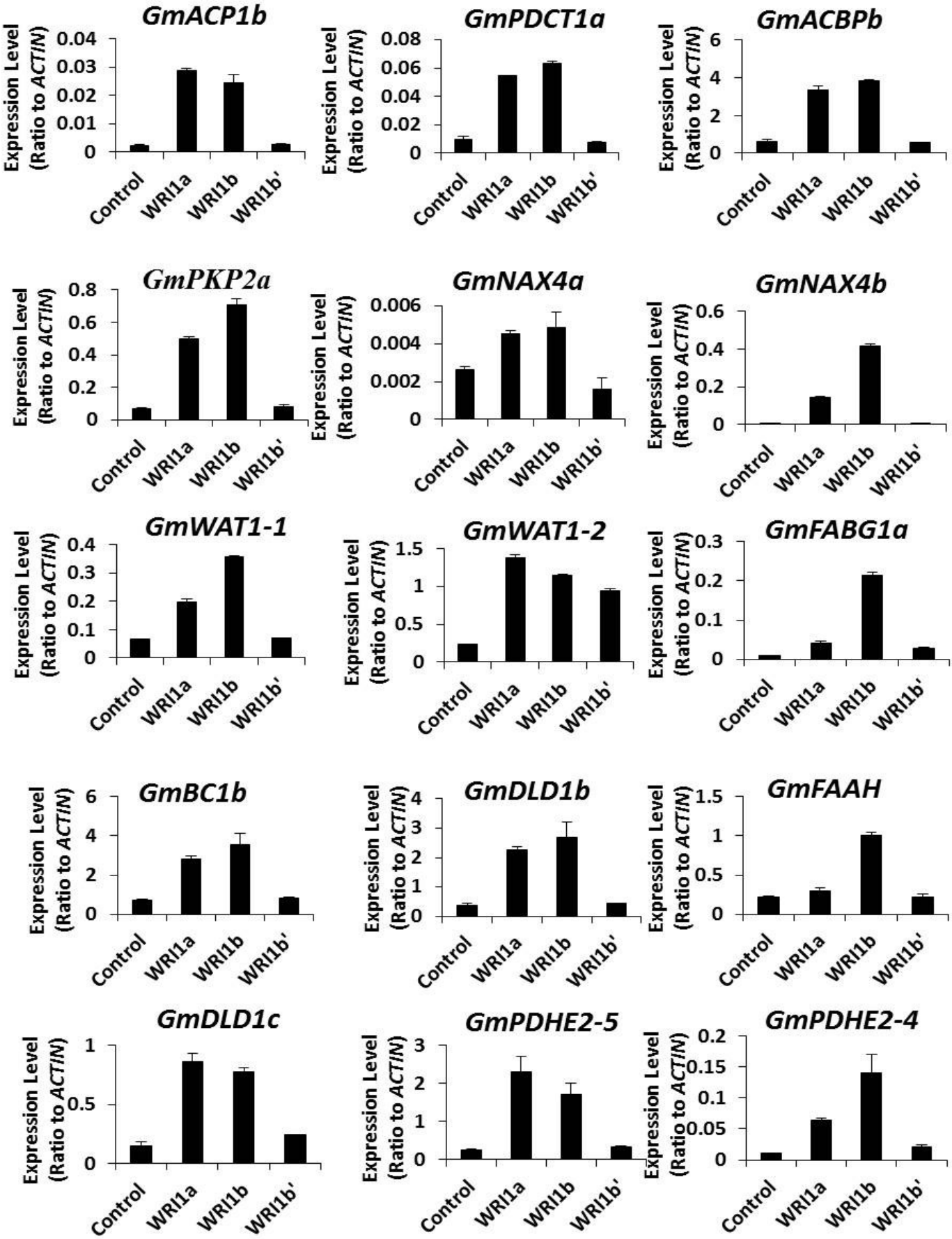

GmACP1b
GmPDCT1a
GmACBPb
GmPKP2a
GmNAX4a
GmNAX4b
GmWAT1-1
GmWAT1-2
GmFABG1a
GmBC1b
GmDLD1b
GmFAAH
GmDLD1c
GmPDHE2-5
GmPDHE2-4
Expression Level (Ratio to ACTIN)
Control
WRI1a
WRI1b
WRI1b'

Figure S7. Quantitative PCR confirmation of gene regulation by GmWRI1 in soybean hairy roots.

ACP1b:Glyma15g098500 (Glyma15g10520)
PDCT1a:Glyma08g213100 (Glyma08g22750)
ABCPb:Glyma09g214500 (Glyma09g34770)
PKP2a:Glyma09g126300 (Glyma09g23150)
Annexin-like protein RJ4(NAX4)
NAX4a:Glyma13g199500 (Glyma13g26960)
NAX4b:Glyma11g153800 (Glyma11g21480)
WAT1-1: Glyma19g173800 (Glyma19g35720)
WAT1-2:Glyma04g251000 (Glyma04g43000)
FABG1:3-oxoacyl-[acyl-carrier-protein] reductase 1
FABG1a:Glyma11g248000 (Glyma11g37320)
BC:Biotin carboxylase 1
BC1b:Glyma08g027600(Glyma08g03120)
DLD1:Dihydrolipoyl dehydrogenase 1
DLD1b:Glyma17g032300 (Glyma17g03560)
DLD1c:Glyma15g143100 (Glyma15g15310)
PDHE2：Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenas
PDHE2-5:Glyma10G215400 (Glyma10g35960)
PDHE2-4:Glyma.20g176300 (Glyma20g24830)
FAAH:Fatty acid amide hydrolase
FAAH:Glyma08g003100 (Glyma08g00535)
All data are expressed as mean ± SD from at least three biological duplicates. * $P < 0.05$ and ** $P < 0.01$ by Student's *t* test for significant difference.

Figure S8

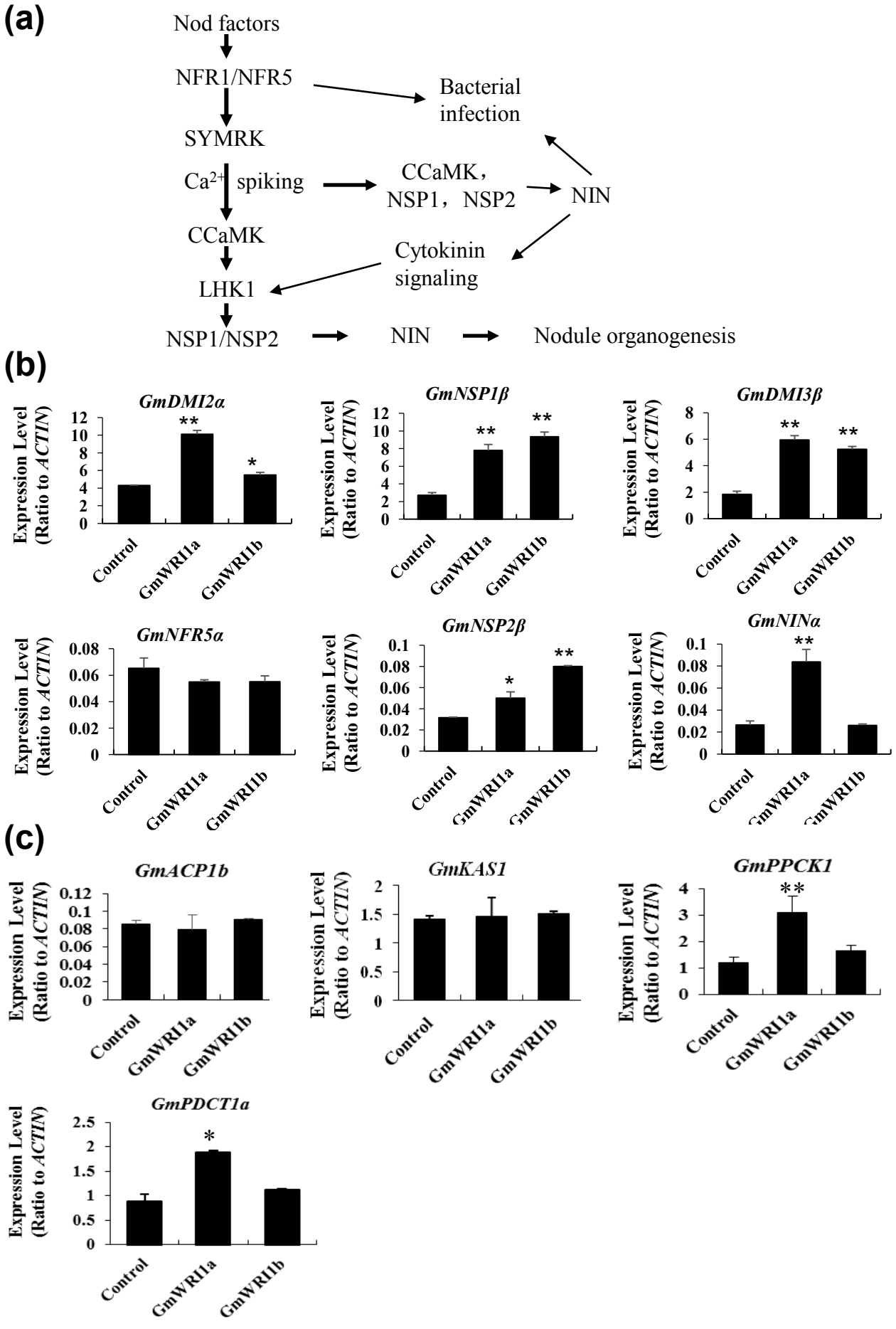

(a)
Nod factors
NFR1/NFR5
Bacterial infection
SYMRK
Ca2+ spiking
CCaMK, NSP1, NSP2
NIN
CCaMK
Cytokinin signaling
LHK1
NSP1/NSP2
NIN
Nodule organogenesis
(b)
GmDMI2α
GmNSP1β
GmDMI3β
GmNFR5α
GmNSP2β
GmNINα
Expression Level (Ratio to ACTIN)
Control
GmWRI1a
GmWRI1b
(c)
GmACP1b
GmKAS1
GmPPCK1
GmPDCT1a

Figure S8. Effects of *GmWRI1a* and *b* overexpression on soybean nodulation and glycolysis and lipid metabolism.

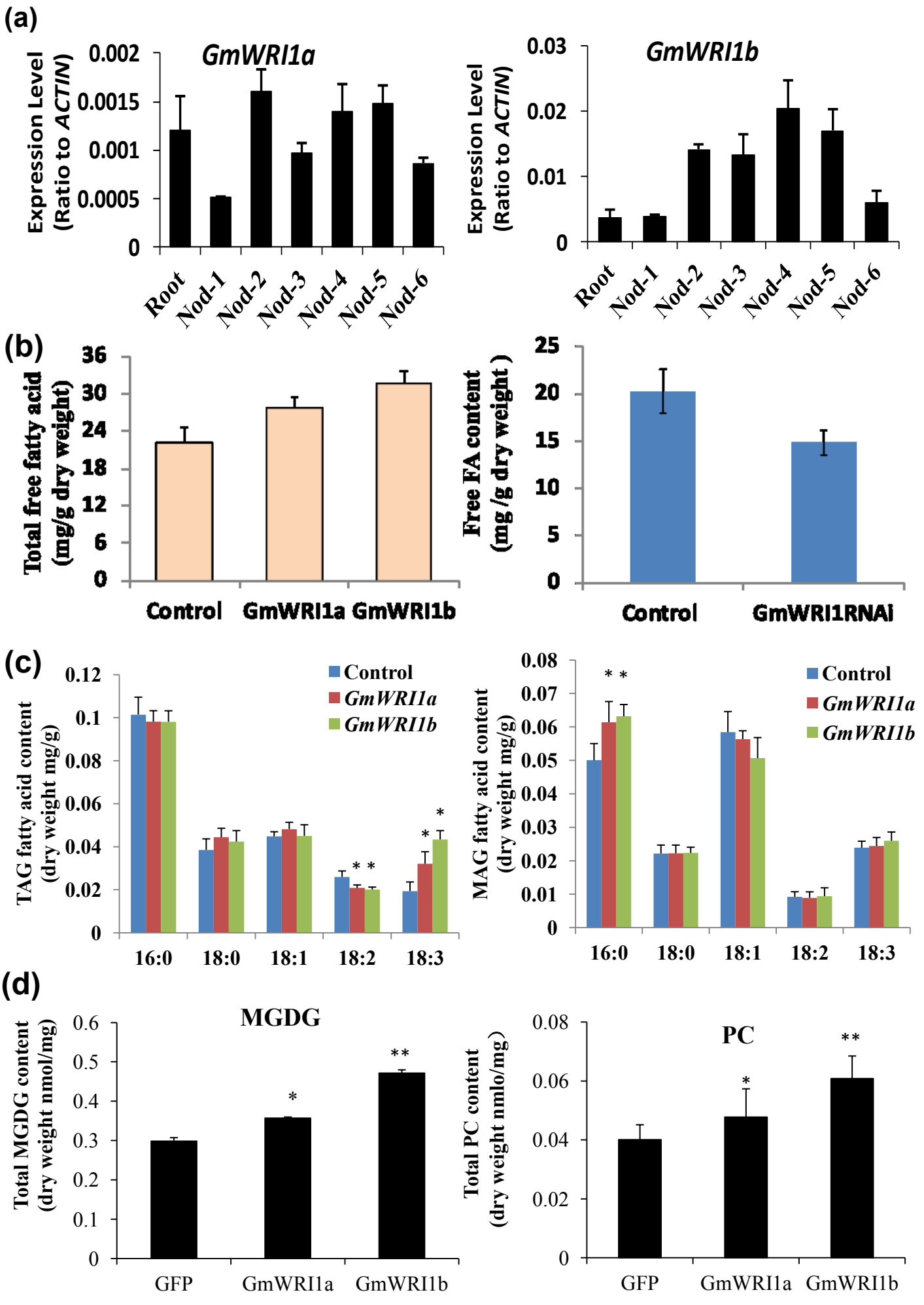
(a) Schematic of nodulation signal pathway.

(b) qRT-PCR verification of expression levels of early nodulation genes.

(c) qRT-PCR verification of expression levels of glycolisis and lipid metabolism gene.

All data are expressed as mean ± SD from at least three biological duplicates. * $P < 0.05$ and ** $P < 0.01$ by Student's *t* test for significant difference.

Figure S9


(a)
GmWRI1a
Expression Level (Ratio to ACTIN)
0.002
0.0015
0.001
0.0005
0
Root
Nod-1
Nod-2
Nod-3
Nod-4
Nod-5
Nod-6
GmWRI1b
0.03
0.02
0.01
(b)
Total free fatty acid (mg/g dry weight)
36
30
24
18
12
6
Control
GmWRI1a
GmWRI1b
Free FA content (mg /g dry weight)
25
20
15
10
5
GmWRI1RNAi
(c)
TAG fatty acid content (dry weight mg/g)
0.12
0.1
0.08
0.06
0.04
0.02
Control
GmWRI1a
GmWRI1b
16:0
18:0
18:1
18:2
18:3
MAG fatty acid content (dry weight mg/g)
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
(d)
MGDG
Total MGDG content (dry weight nmol/mg)
0.6
0.5
0.4
0.3
0.2
0.1
GFP
GmWRI1a
GmWRI1b
PC
Total PC content (dry weight nmlo/mg)
0.08
0.06
0.04
0.02

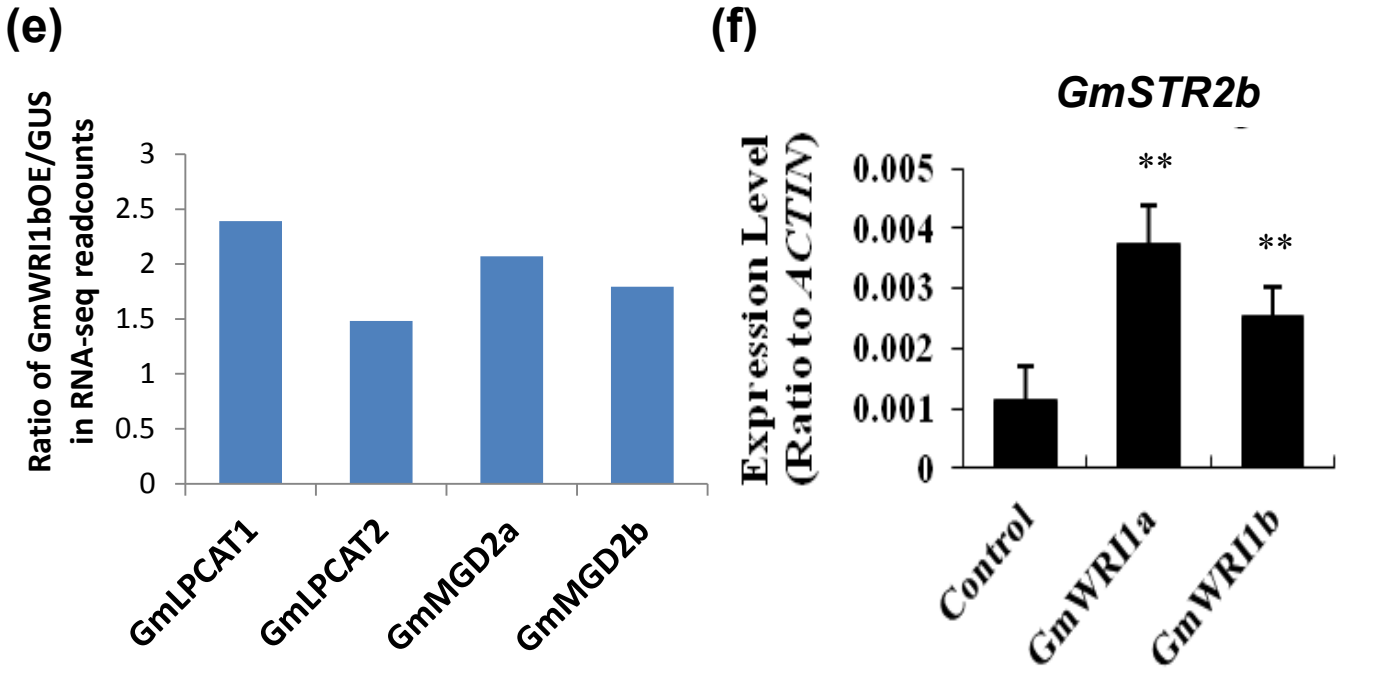


Figure S9. Accumulation of lipids and expression patterns of soybean lipid synthesis –related genes at soybean nodule developmental stages.

(a) qRT-PCR analysis of *GmWRI1* expression.

(b) Contents of free fatty acids in soybean nodules of *GmWRI1a* or *b*-overexpressing and *GmWRI1RNAi* hairy roots, as compared with these nodules from *GUS*-expressing hairy roots (as a control).

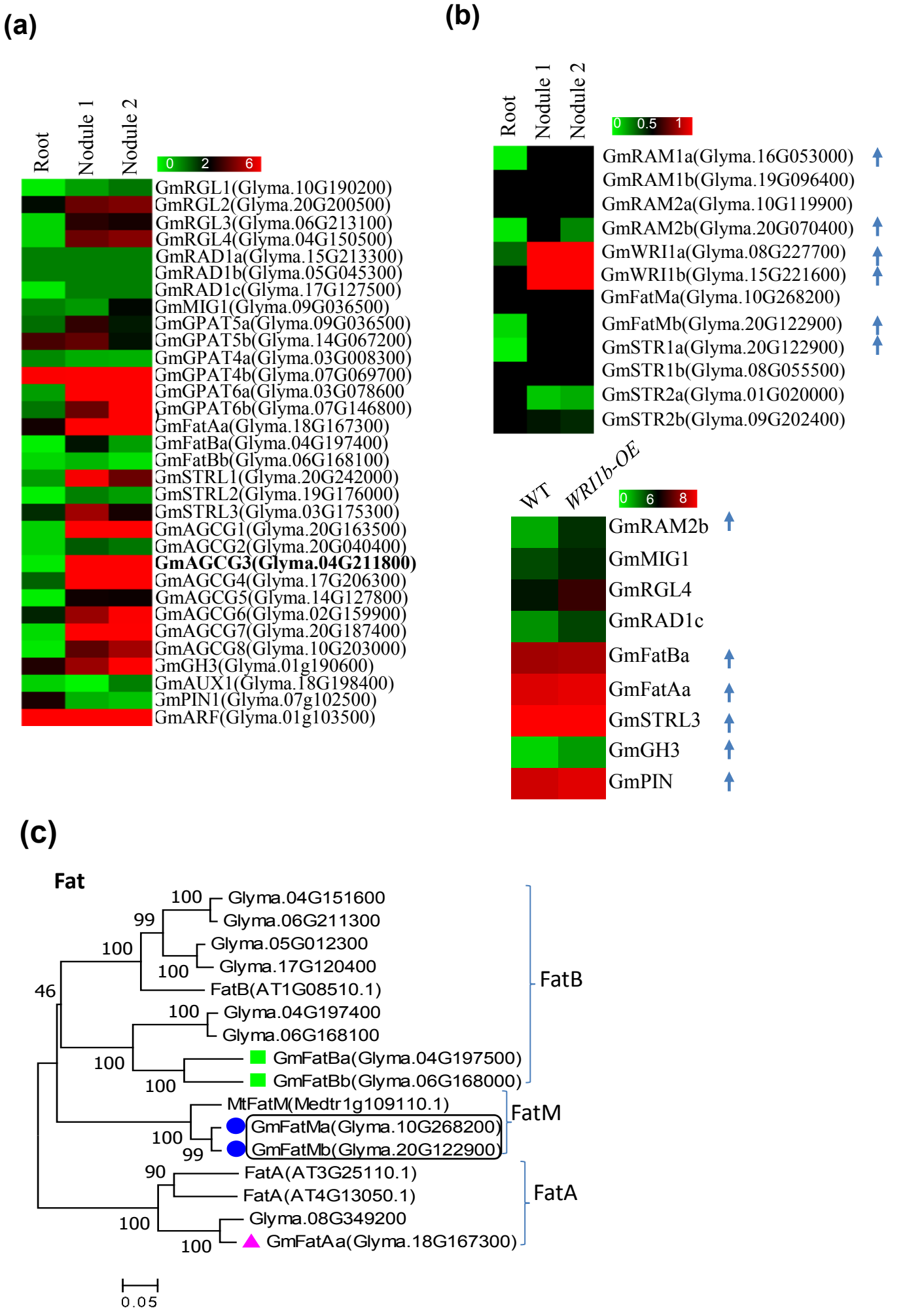
(c) Fatty acid contents in TAGs and MAGs from nodules overexpressing *GmWRI1a* and *GmWRI1b*.

(d) Total MGDG and PC contents in the nodules overexpressing *GmWRI1a* and *GmWRI1b*.

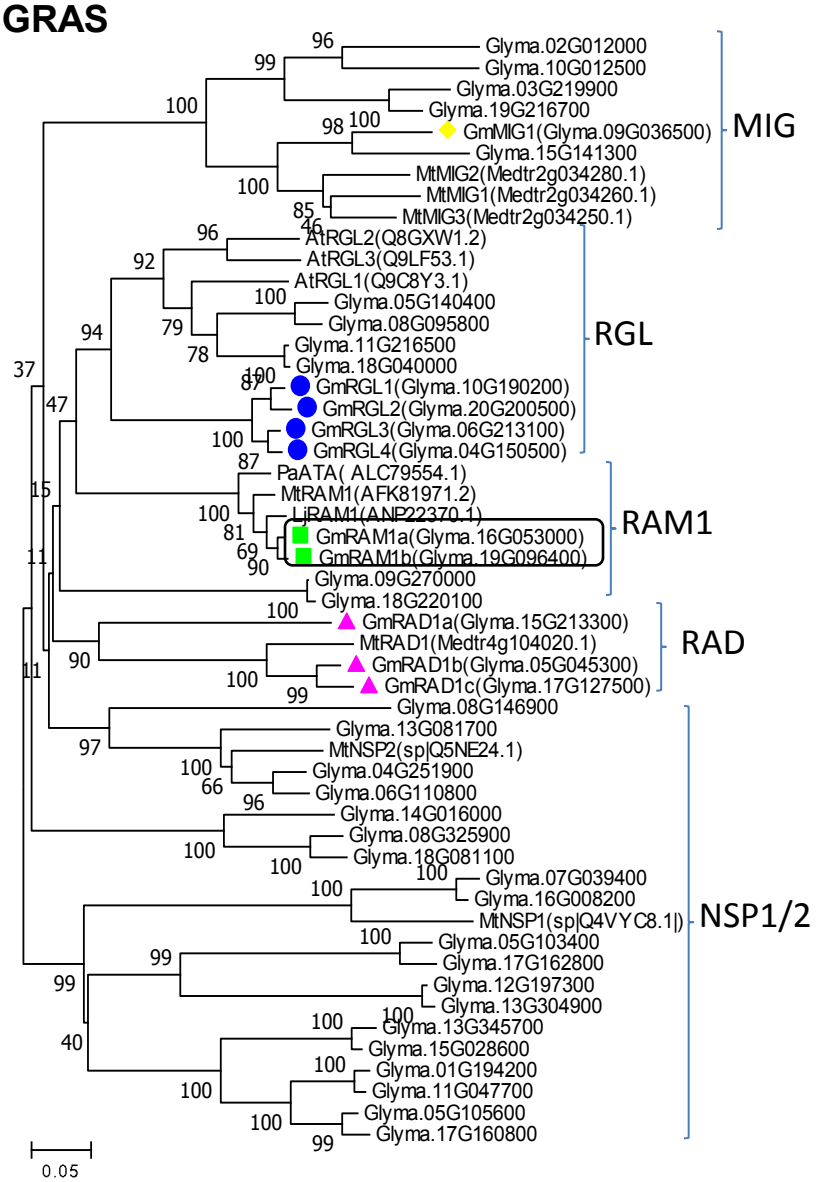
(e) Up-regulation of PC and MGDG synthesis genes lysophosphatidylcholine acyltransferase 1 (LPCAT1)(Glyma17g14070) and LPCAT2(Glyma05g03510) and Type B MGDG synthase 2a (MGD2a) (Glyma17g11720) and GmMGD2b (Glyma13g23150) in GmWRI1b OE hairy roots as compared with GUS control.

(f) qRT-PCR verification of expression levels of lipid transporter gene GmSTR2b (Glyma09G202400).

Figure S10


(a)
Root
Nodule 1
Nodule 2
0 2 6
GmRGL1(Glyma.10G190200)
GmRGL2(Glyma.20G200500)
GmRGL3(Glyma.06G213100)
GmRGL4(Glyma.04G150500)
GmRAD1a(Glyma.15G213300)
GmRAD1b(Glyma.05G045300)
GmRAD1c(Glyma.17G127500)
GmMIG1(Glyma.09G036500)
GmGPAT5a(Glyma.09G036500)
GmGPAT5b(Glyma.14G067200)
GmGPAT4a(Glyma.03G008300)
GmGPAT4b(Glyma.07G069700)
GmGPAT6a(Glyma.03G078600
GmGPAT6b(Glyma.07G146800)
GmFatAa(Glyma.18G167300)
GmFatBa(Glyma.04G197400)
GmFatBb(Glyma.06G168100)
GmSTRL1(Glyma.20G242000)
GmSTRL2(Glyma.19G176000)
GmSTRL3(Glyma.03G175300)
GmAGCG1(Glyma.20G163500)
GmAGCG2(Glyma.20G040400)
GmAGCG3(Glyma.04G211800)
GmAGCG4(Glyma.17G206300)
GmAGCG5(Glyma.14G127800)
GmAGCG6(Glyma.02G159900)
GmAGCG7(Glyma.20G187400)
GmAGCG8(Glyma.10G203000)
GmGH3(Glyma.01g190600)
GmAUX1(Glyma.18G198400)
GmPIN1(Glyma.07g102500)
GmARF(Glyma.01g103500)
(b)
Root
Nodule 1
Nodule 2
0 0.5 1
GmRAM1a(Glyma.16G053000)
GmRAM1b(Glyma.19G096400)
GmRAM2a(Glyma.10G119900)
GmRAM2b(Glyma.20G070400)
GmWRI1a(Glyma.08G227700)
GmWRI1b(Glyma.15G221600)
GmFatMa(Glyma.10G268200)
GmFatMb(Glyma.20G122900)
GmSTR1a(Glyma.20G122900)
GmSTR1b(Glyma.08G055500)
GmSTR2a(Glyma.01G020000)
GmSTR2b(Glyma.09G202400)
WT
WRI1b-OE
0 6 8
GmRAM2b
GmMIG1
GmRGL4
GmRAD1c
GmFatBa
GmFatAa
GmSTRL3
GmGH3
GmPIN
(c)
Fat
100
Glyma.04G151600
99
Glyma.06G211300
100
Glyma.05G012300
100
Glyma.17G120400
46
FatB(AT1G08510.1)
100
Glyma.04G197400
Glyma.06G168100
100
GmFatBa(Glyma.04G197500)
100
GmFatBb(Glyma.06G168000)
FatB
MtFatM(Medtr1g109110.1)
GmFatMa(Glyma.10G268200)
100
GmFatMb(Glyma.20G122900)
99
FatM
90
FatA(AT3G25110.1)
FatA(AT4G13050.1)
100
Glyma.08G349200
100
GmFatAa(Glyma.18G167300)
FatA
0.05

(d)


GRAS
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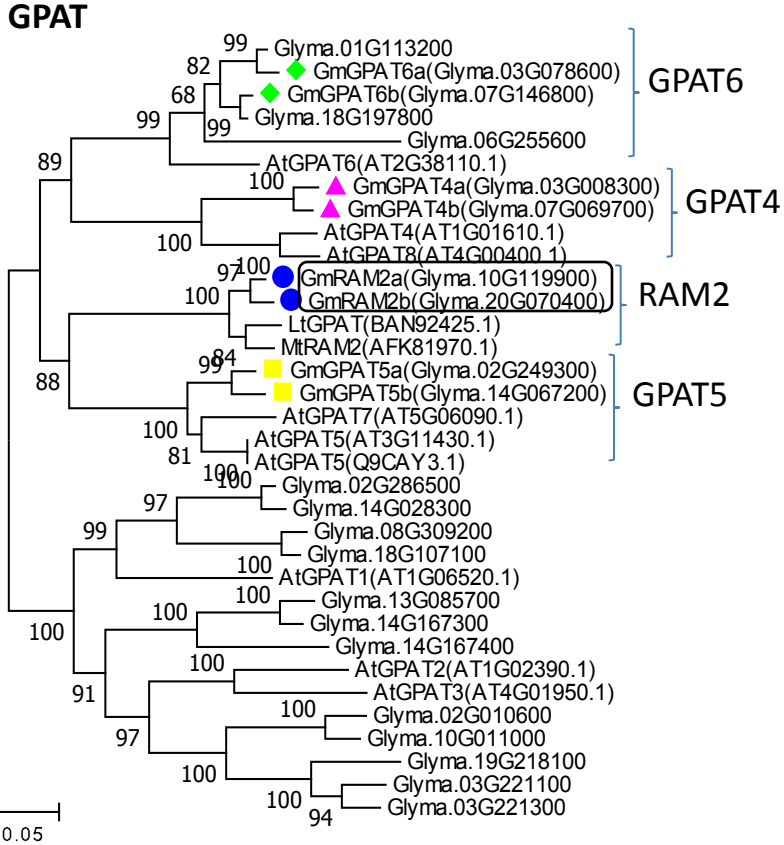
Glyma.10G012500
Glyma.03G219900
Glyma.19G216700
GmMIG1(Glyma.09G036500)
Glyma.15G141300
MtMIG2(Medtr2g034280.1)
MtMIG1(Medtr2g034260.1)
MtMIG3(Medtr2g034250.1)
MIG
AtRGL2(Q8GXW1.2)
AtRGL3(Q9LF53.1)
AtRGL1(Q9C8Y3.1)
Glyma.05G140400
Glyma.08G095800
Glyma.11G216500
Glyma.18G040000
GmRGL1(Glyma.10G190200)
GmRGL2(Glyma.20G200500)
GmRGL3(Glyma.06G213100)
GmRGL4(Glyma.04G150500)
RGL
PaATA(ALC79554.1)
MtRAM1(AFK81971.2)
LjRAM1(ANP22370.1)
GmRAM1a(Glyma.16G053000)
GmRAM1b(Glyma.19G096400)
RAM1
Glyma.09G270000
Glyma.18G220100
GmRAD1a(Glyma.15G213300)
MtRAD1(Medtr4g104020.1)
GmRAD1b(Glyma.05G045300)
GmRAD1c(Glyma.17G127500)
RAD
Glyma.08G146900
Glyma.13G081700
MtNSP2(sp|Q5NE24.1)
Glyma.04G251900
Glyma.06G110800
Glyma.14G016000
Glyma.08G325900
Glyma.18G081100
Glyma.07G039400
Glyma.16G008200
MtNSP1(sp|Q4VYC8.1|)
Glyma.05G103400
Glyma.17G162800
Glyma.12G197300
Glyma.13G304900
Glyma.13G345700
Glyma.15G028600
Glyma.01G194200
Glyma.11G047700
Glyma.05G105600
Glyma.17G160800
NSP1/2
0.05

(e)


GPAT
Glyma.01G113200
GmGPAT6a(Glyma.03G078600)
GmGPAT6b(Glyma.07G146800)
Glyma.18G197800
Glyma.06G255600
GPAT6
AtGPAT6(AT2G38110.1)
GmGPAT4a(Glyma.03G008300)
GmGPAT4b(Glyma.07G069700)
AtGPAT4(AT1G01610.1)
AtGPAT8(AT4G00400.1)
GPAT4
GmRAM2a(Glyma.10G119900)
GmRAM2b(Glyma.20G070400)
LtGPAT(BAN92425.1)
MtRAM2(AFK81970.1)
RAM2
GmGPAT5a(Glyma.02G249300)
GmGPAT5b(Glyma.14G067200)
AtGPAT7(AT5G06090.1)
AtGPAT5(AT3G11430.1)
AtGPAT5(Q9CAY3.1)
GPAT5
Glyma.02G286500
Glyma.14G028300
Glyma.08G309200
Glyma.18G107100
AtGPAT1(AT1G06520.1)
Glyma.13G085700
Glyma.14G167300
Glyma.14G167400
AtGPAT2(AT1G02390.1)
AtGPAT3(AT4G01950.1)
Glyma.02G010600
Glyma.10G011000
Glyma.19G218100
Glyma.03G221100
Glyma.03G221300
0.05

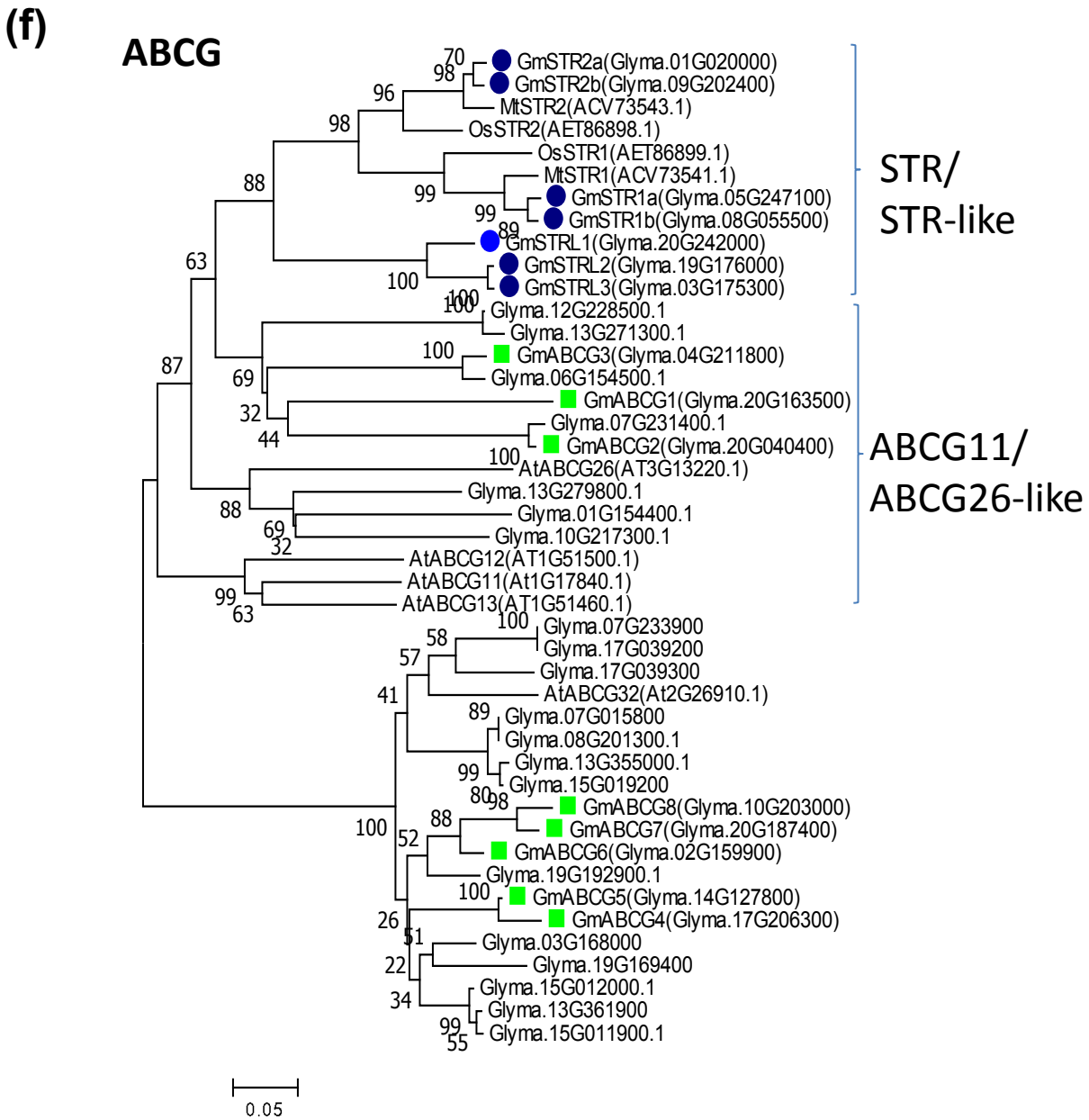


Figure S10. Identification and transcriptional analysis of FatB, GPAT, GRAS transcription factors RAM1 and ABCG transporter STR1/2.

(a) Heatmap analysis of soybean homologue genes for Medicago RAM1, RAM2, WRI1, STR, and FatM.

(**b**) Expression of ortholog genes (g) for *M. truncatula* RAM1, RAM2, WRI1, STR1/2, and FatM in root and nodule and genes with WRI1-binding cis-elements related to auxin modification, transport, signaling, or 2-MAG synthesis or transport in WT and *GmWRI1bOE* line.

(**c**) Phylogenetic identification of soybean FatB and FatA genes homology to Medicago and Lotus or other fatty acyl binding protein thioesterase (Fat)

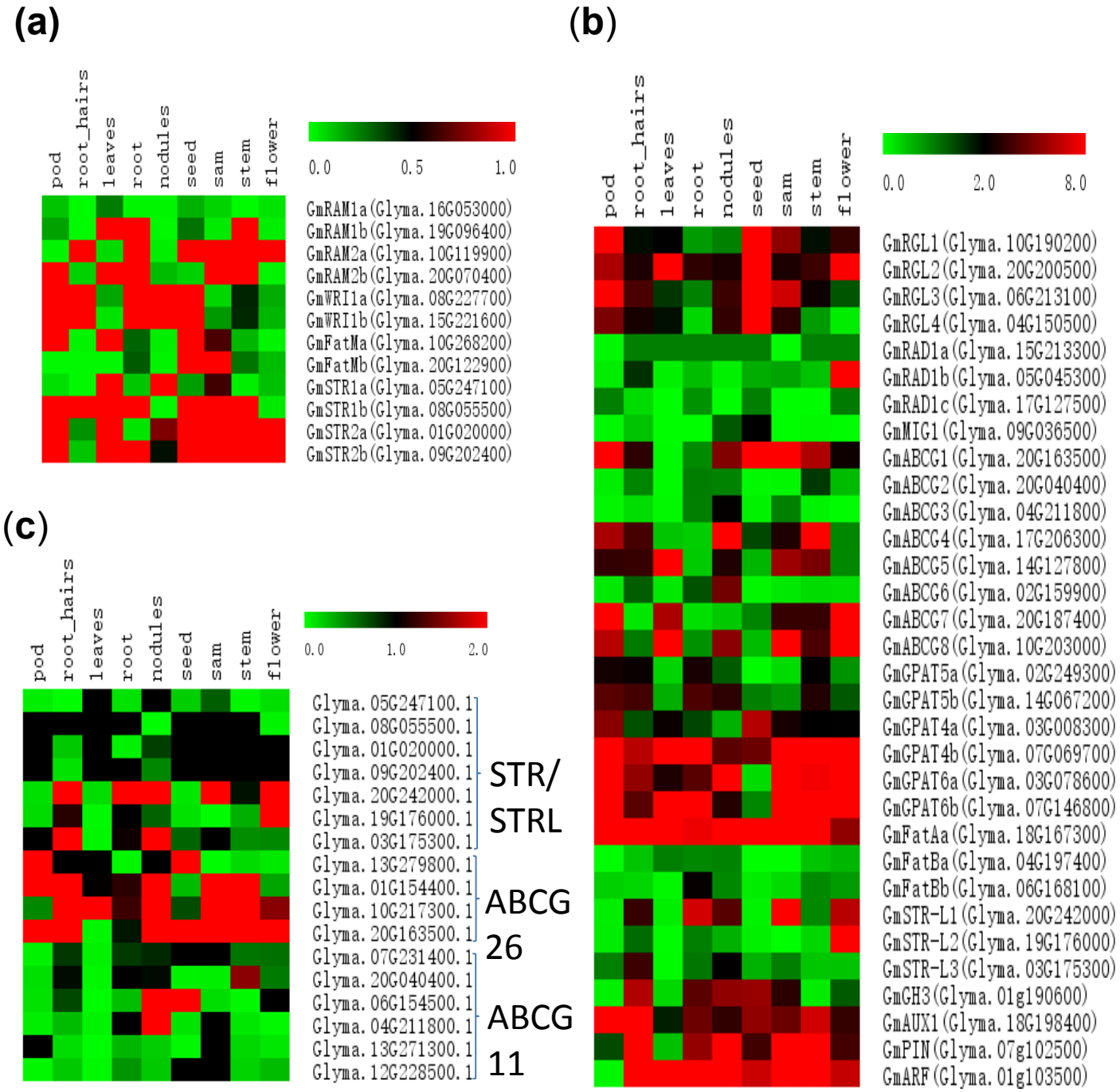
(**d**) Phylogenetic identification of soybean GRAS genes homology to Medicago and Lotus or other GPATs.

(**e**) Phylogenetic identification of soybean GPAT genes homology to Medicago and Lotus or other GPATs.

(**f**) Phylogenetic identification of soybean ABCG transporter genes homology to Medicago and Lotus or other STR1/2 or lipid transporters ABCG11, ABCG26 . .

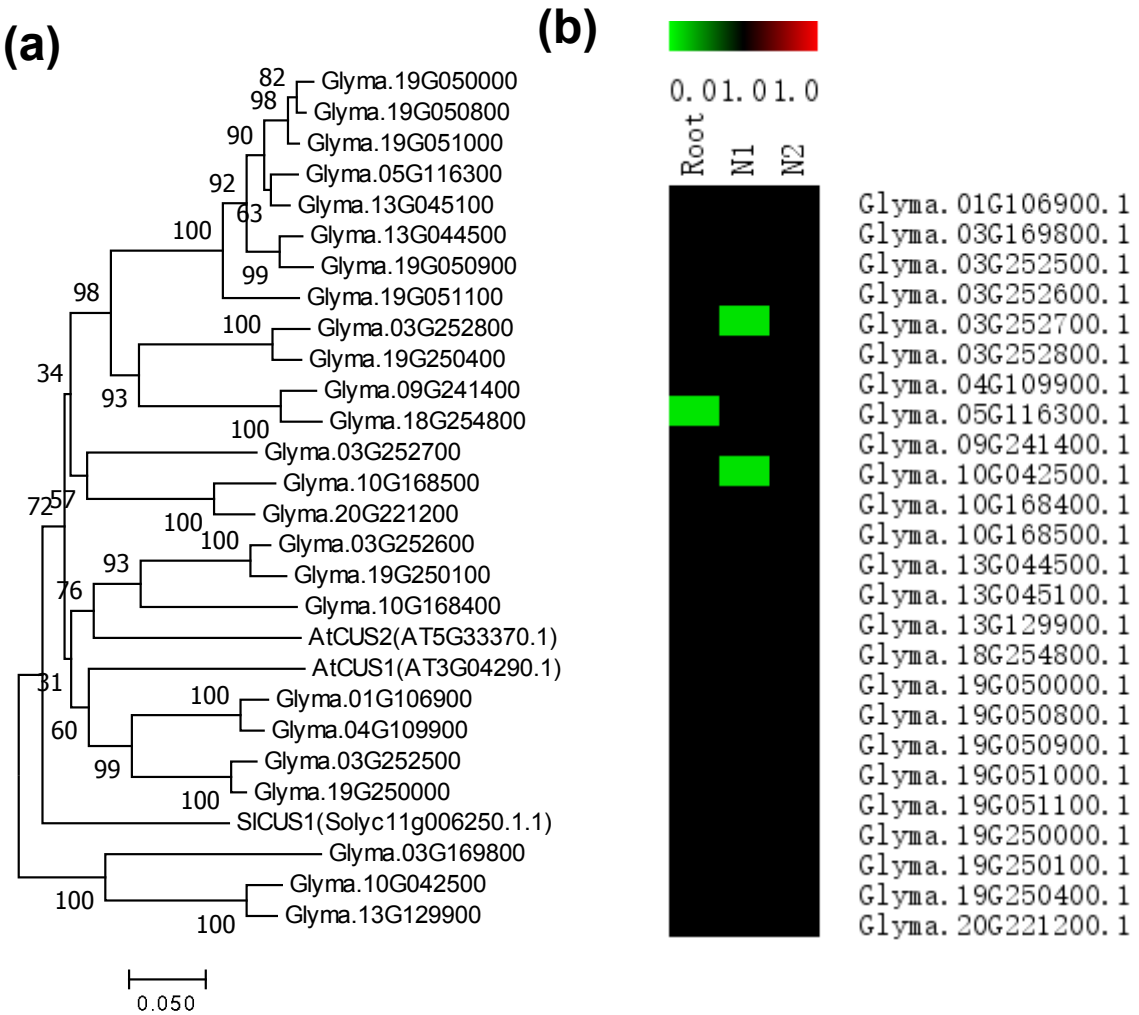
The alignment was generated using Clustal W and the unrooted phylogram was constructed with MEGA6 software using the neighbor-joining method**.**

Figure S11



Supplemental figure S11. The heat map analysis of tissue expression patterns of ortholog genes (**a**), other homology genes (**b**) for M. truncatula RAM1, RAM2, WRI1, STR1/2, and FatM. (**c**) is the tissue expression of ABCG11, ABCG26, RGL, RAD, and STR-like (STRL).

Figure S12



Supplementary figure12. The phylogenic tree (**a**) and heat map analysis (**b**) in roots and nodules of CUS (cutin synthase). The alignment was generated using Clustal and the unrooted phylogram was constructed with MEGA6 software using the neighbor-joining method. The heat map was made by MeV software.

Figure S13

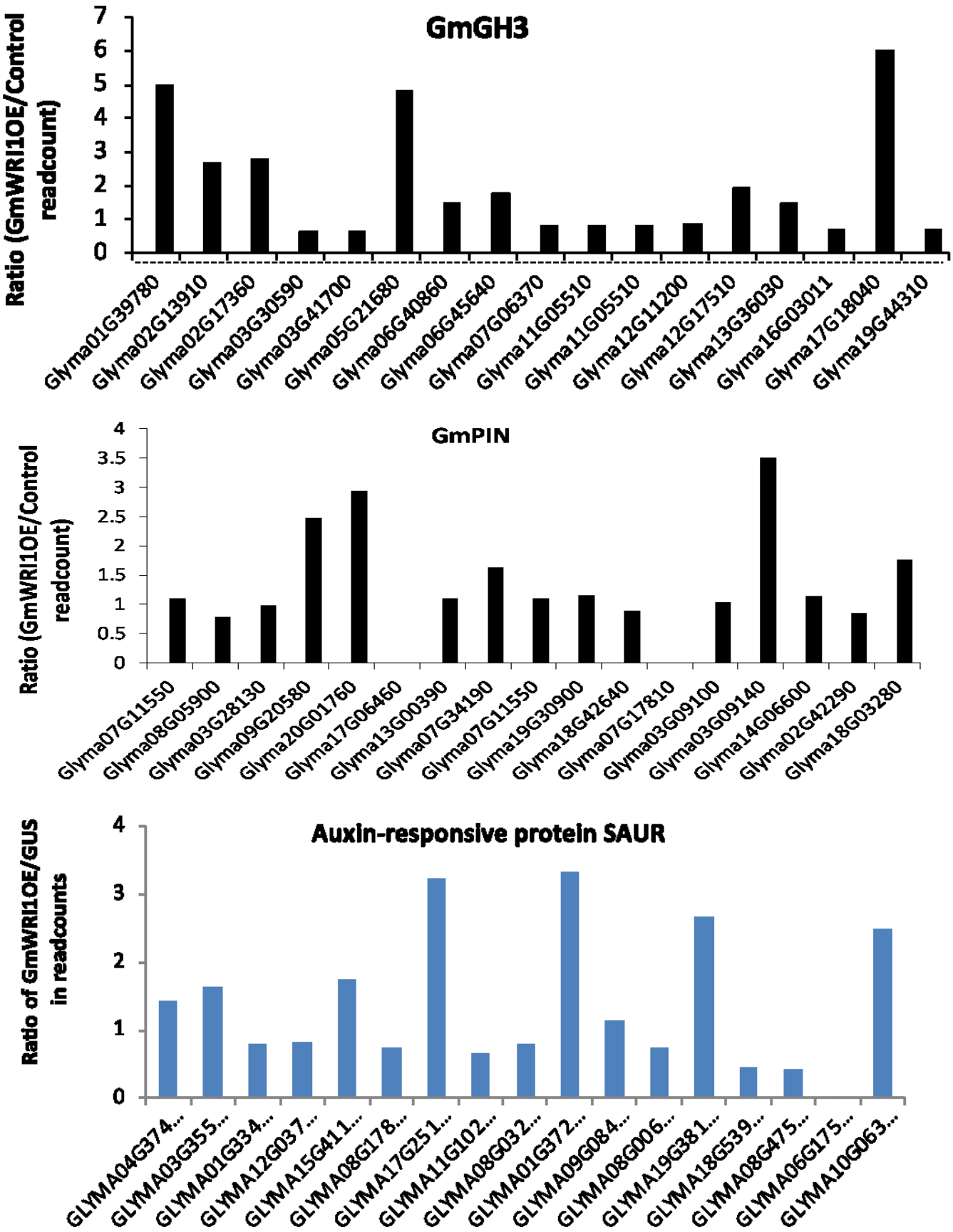


Figure S13. Expression level of *GmGH3*，*GmPIN*，and *Auxin-responsive protein SAUR* genes in *GmWRI1b* overexpression (*GmWRI1bOE)* hairy roots, as compared with control (*GUS*) hairy roots.

Figure S14

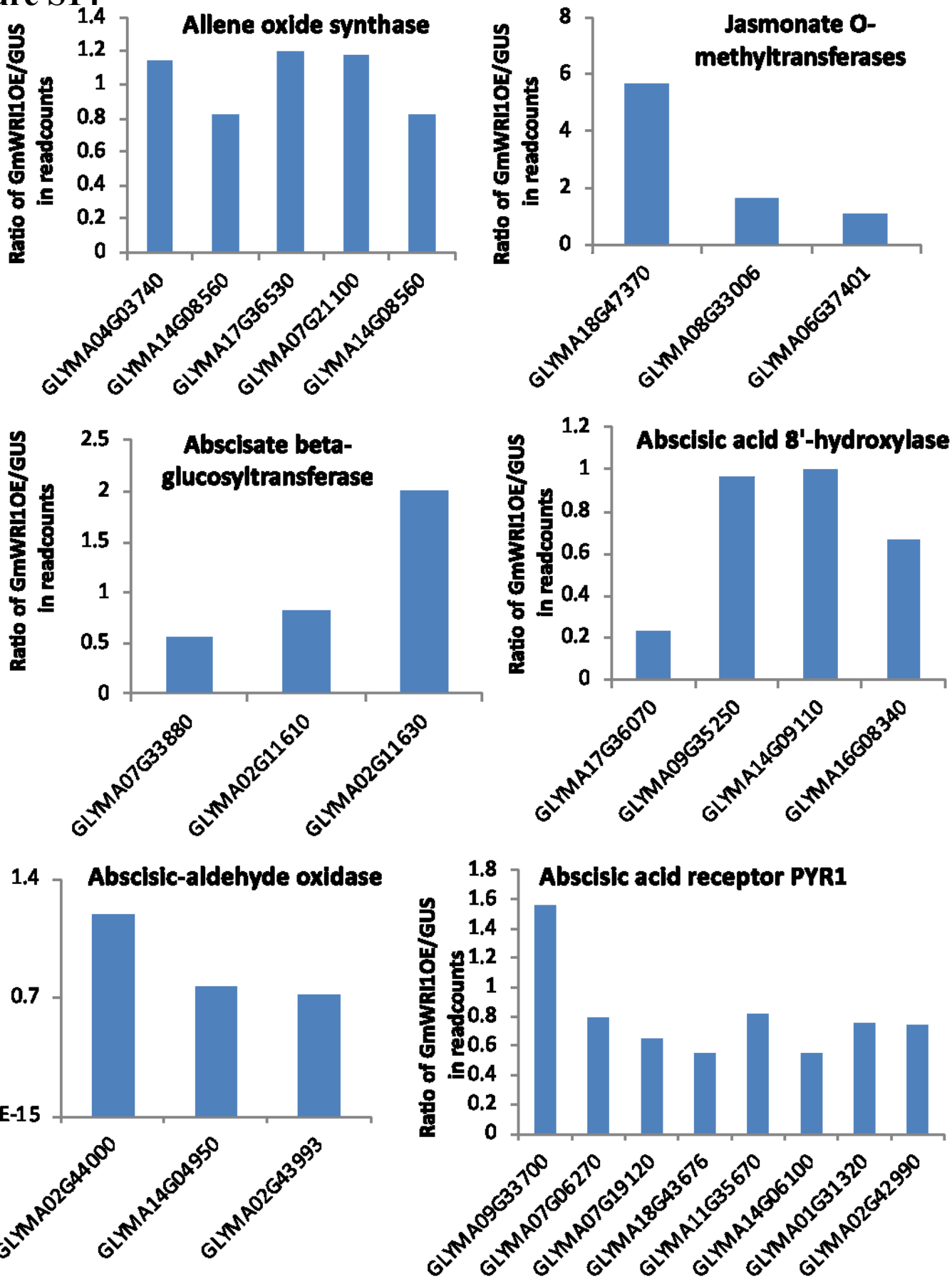


Figure S14. Expression level of jasmonate biosynthesis and abscisic acid biosynthesis and catabolic genes in *GmWRI1b* overexpression (*GmWRI1bOE)* hairy roots, as compared with control (*GUS*) hairy roots.

Figure S15

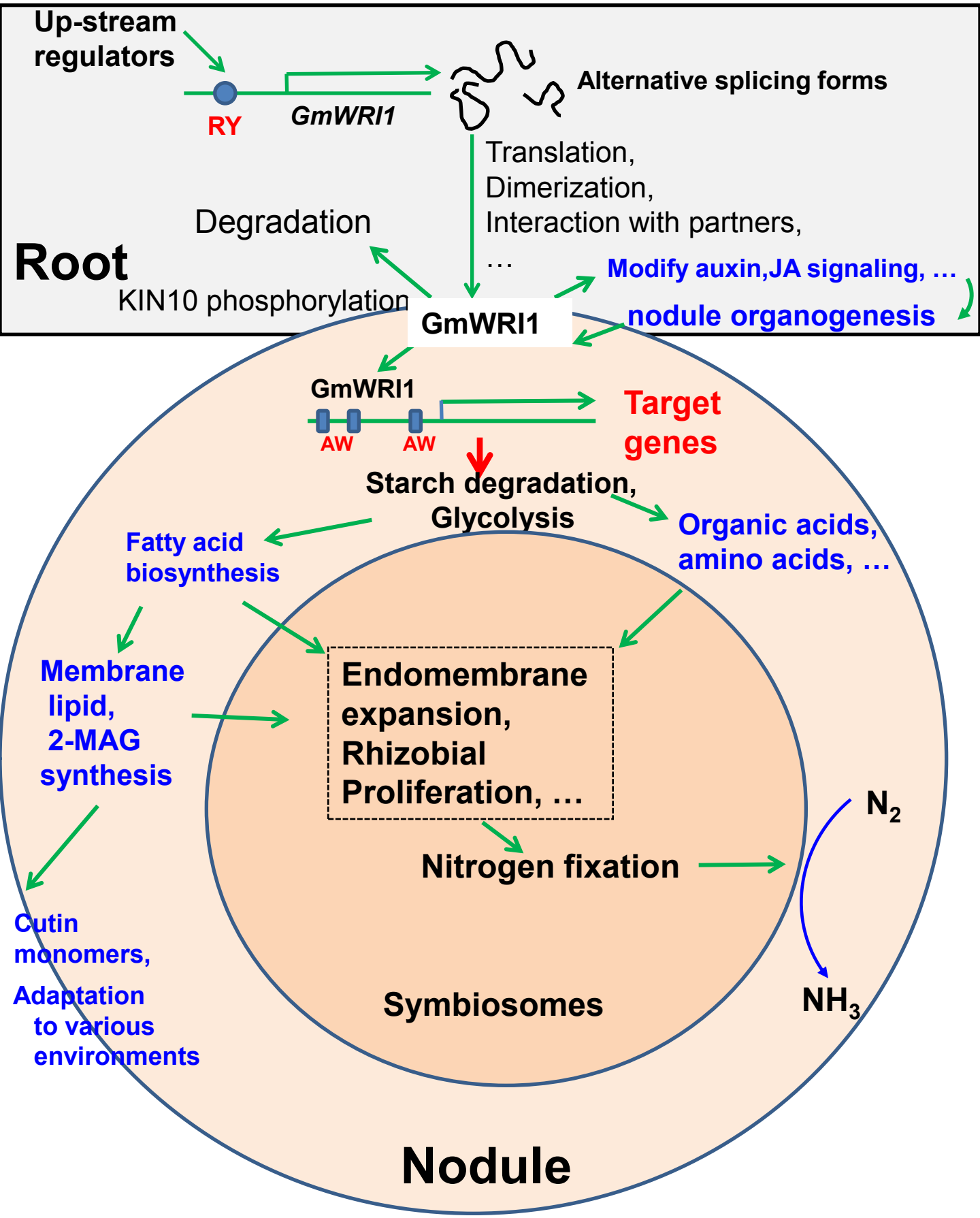


Figure S15. Proposed functions for GmWRI1s in soybean nodulation