

Expression analysis of *PIN* genes in root tips and nodules of *Lotus japonicus*

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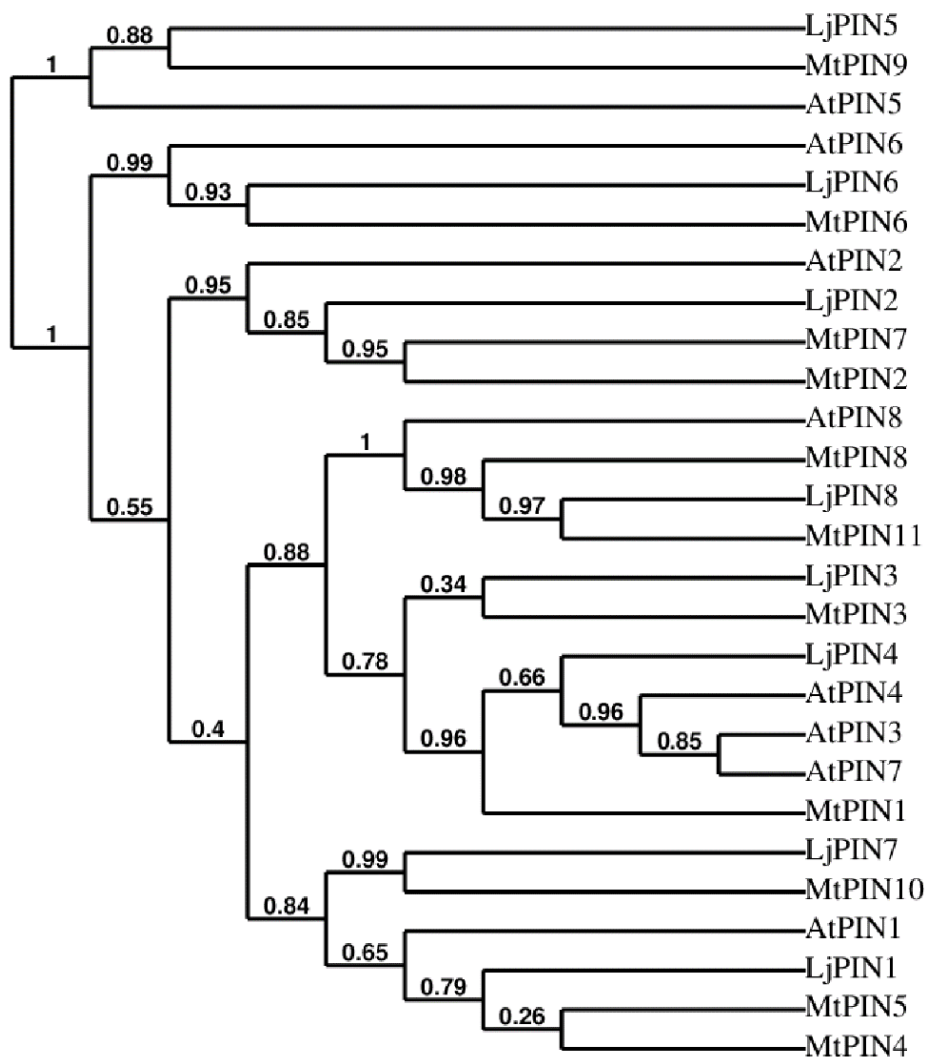


Figure S1. Cladogram presenting the phylogenetic relationship of PIN proteins from *L. japonicus* (Lj), *M. truncatula* (Mt) and *A. thaliana* (At).

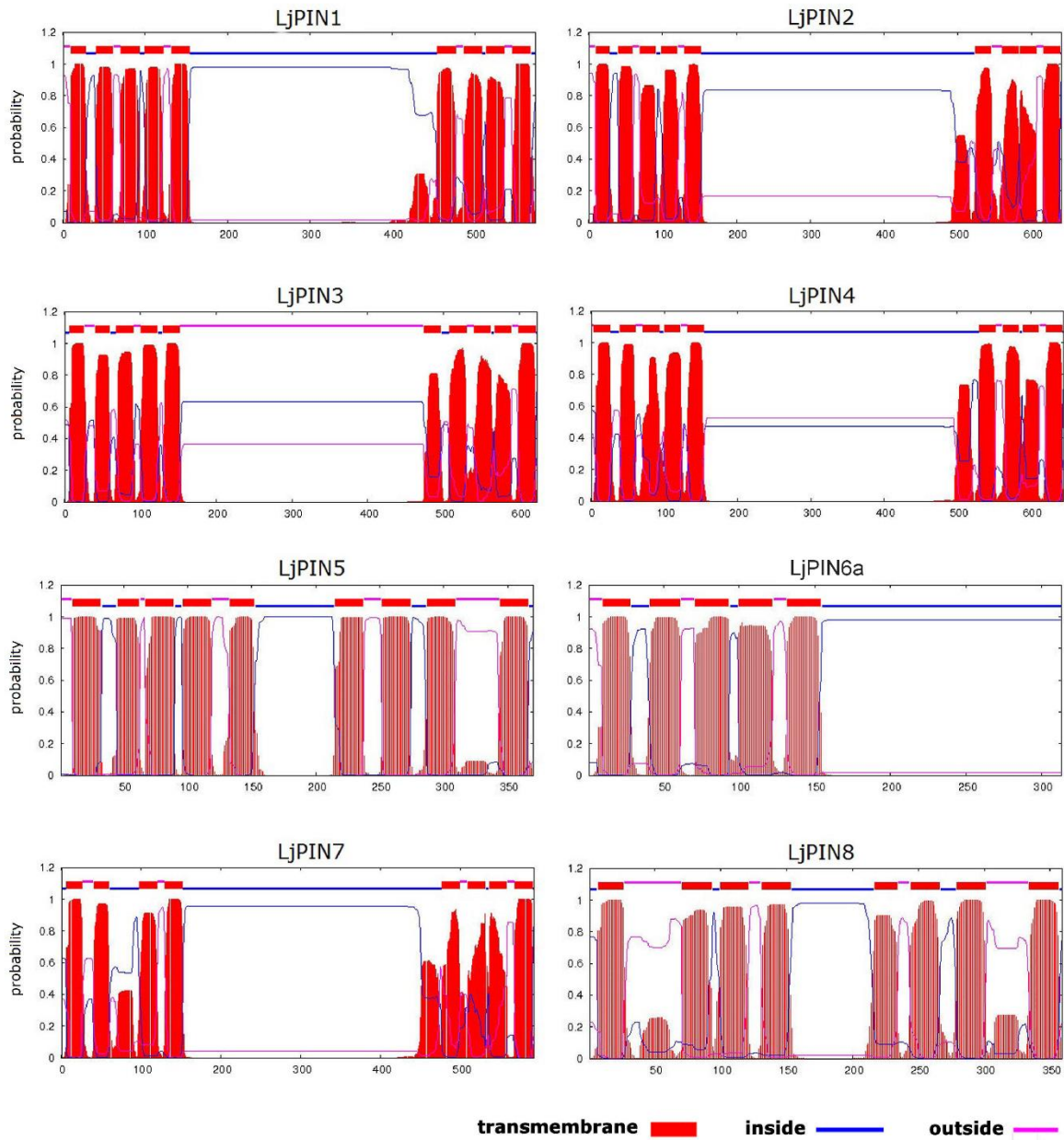


Figure S2. Transmembrane topology of *L. japonicus* PIN proteins. Red peaks represent transmembrane helices.

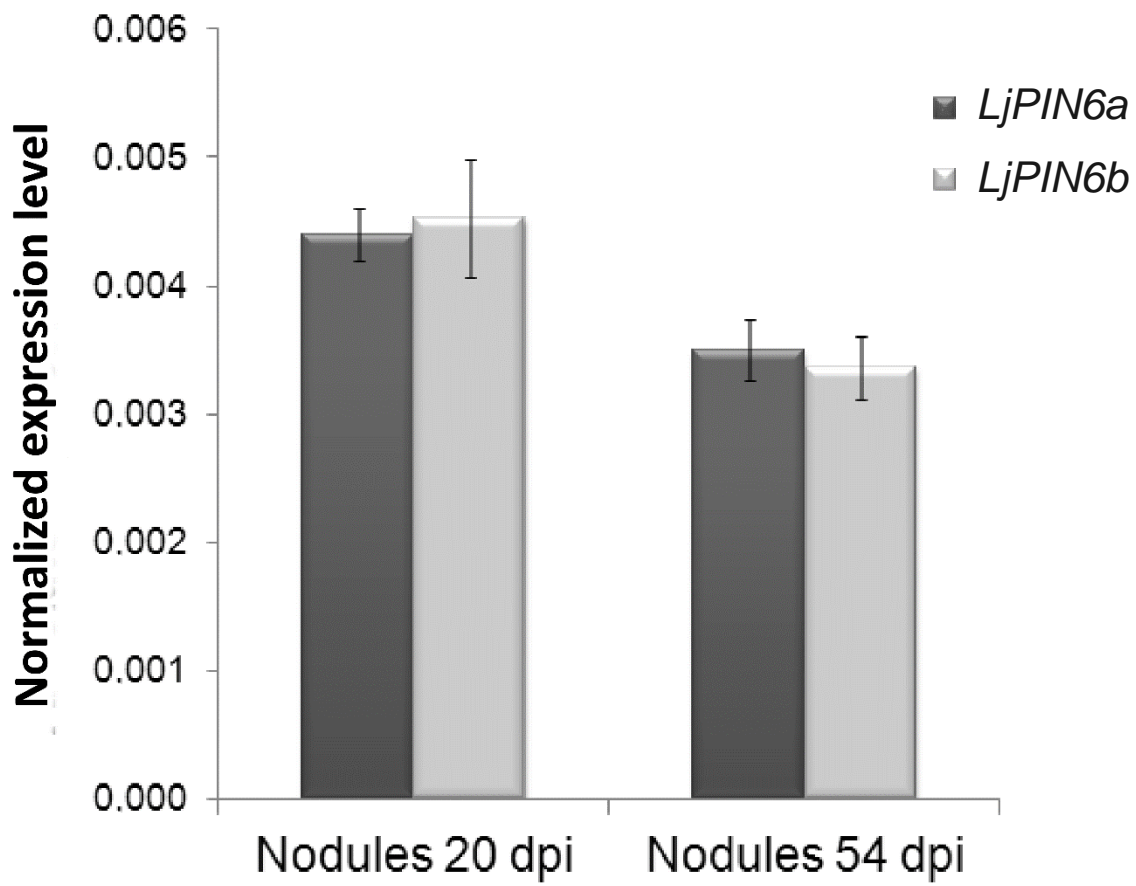


Figure S3. Normalized expression level of *LjPIN6a* and *LjPIN6b* in root nodules. Mean values (\pm SE) are derived from three biological replicates, for which three individual qPCR reactions were performed.

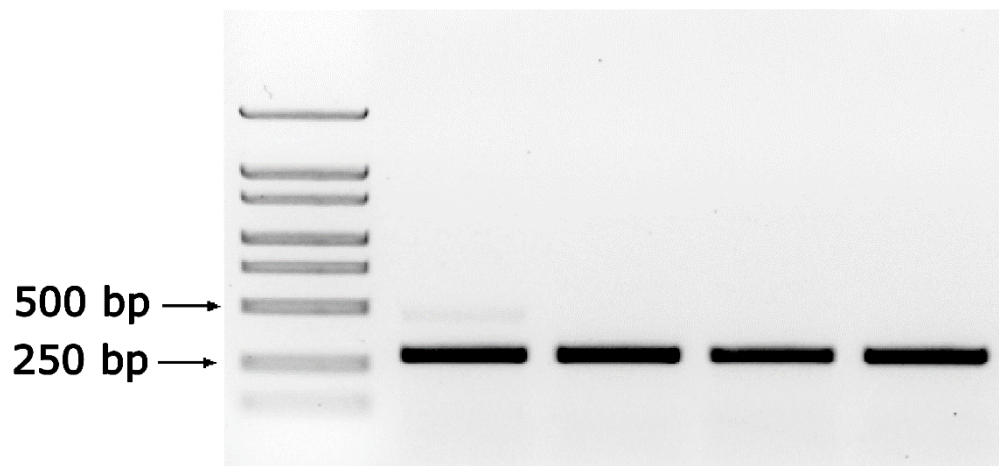


Figure S4. The electrophoretic separation of LjPIN6 fragment amplification products. bp- base pairs.

A

LjPIN6 – complete coding sequence (CDS)

ATGGTTACAGGAGATGATTTGTACAACGTGATGTGCGCCATGGTGCC
TCTATATTTTCGCAATGCTGGTAGCTTACGCATCAGTGAAATGGTGCA
AAATGTTACCCACAGCAGTGTCCGGCGTCAACCGTTTCGTGGCG
GTGTTTGGCATCCGGTCTCTCTTTCCATTTCAITTCATCAACAATC
CTTATGAGATGGACGCTAAGTTCATCATAGCGGACACGCTCTCCAAG
CTCGTCGTGCTGTTCTTCTCTCTCTGGGCTATTTTTTTGCTTCTG
GATCTCTCGACTGGCTTATCACGCTCTTCTCGCTCGCAACCTTGCCCA
ACACGCTTGTATGGGGATTCTCTACTCCAAGCCATGTACGGCCAA
TTCACGCAAGGCCTCATGGTGCAACTCGTGTACTCCAATGCATCATA
TGGTATACTTACTGTTGTTTCTATTGGAATACAGAGCAGCGACCCTT
TTGATCAAAAACCGAGTCCCTGGGGACACGGCGGCGTCCATAACCAA
AATCGAGGTCGACGGCGACGTGATTTCCCTCGACGGCCACGACGTGC
CACTGCAACCGCAGTCGGAAACCGACACCCACGGCCGATCAGCGT
GCGAATCCGGCGATCAATCTCTCAGCTCCGGACTCAACCTCATCAA
TCGGCAACGCGTTATCACTCCAAGGCATTTCGAATCTACCAACGCC
GAGCACCTGTCTGACGGCGACCCCTCTTTGGATACCCGCGGCGAG
CCCGAGGTTATCCGGATGCGCTCTCCGACGCGTACTCGCTTACGCC
CACGCCGCGGGCGTCAAAATTTCAATGAGACGGAGGTCACGGCGGGA
ACGCCGGTATGGGGGAGGTCTCCGGTGGGCGGGGAGGGTTTCTCG
GCAGATGTCGCCGGGGAAATGCCAAGTAGAAGAGAGACAG**GGATGC**
AAGGACATTACGCAGTCAGATAAAGAAATTAGCTTCAGAGACAAC
ATCAAAGTTCAAATGCCAGGGGAAGAAGCTGCAGACACAACCTGCCTC
GTAACAGAAAAATGCCACATGCTTTTGTATGATAAGGCTTATACTT
ACAGTAGTGGGAAGAAAAC**TTTC**CGCGTAACCCTAATACATATTCTAG
TGTATTAGGACTTGTGGTCTCTAATCTCCTTCAAATGGAACATGGA
AATGCCTAGTCTGATCAAAGCATCTATCAAATCATCTCGGATGCAA
GCCTTGGAAATGGCTATGTTTAGCTTAGGGCTTTTCATGGCCCTCAGC
CTCGTATCATTGCTTGTGGTACCAAAAAGAGCAGCCATGGGAATGGCC
ATTCTGTTTCGTGTGCGGGCCTCTTGTAAATGTCAGCATCCTCCATTGTC
ATTGGATTGAGAGGGGACAAATGCACACAGCAATTGTACAGGCTGC
ACTTCCACAGGGGATTGACATTTGTATTTGCAAGGGAATATGGGC
TGCATCCTGATATTTGAGCACAGGGGTTATCTTTGGCATGCTAGTAT
CCTTACCAATAACTCTCTATATTACATACTTCTTGGCTTTGTA

LjPIN6 – complete protein sequence

MVTGDDLYNVMCAMVPLYFAMLVAYASVKWCKMFTPQQCSGVNRFV
AVFAIPVLSFHIFISINPYEMDAKFIIADTSLKLVVLFLLFLWAIFASFSL
DWLITLFLSLATLNTLVMGIPLLQAMYGFQFTQGLMVQLVVLQCIWYTL
LLFLLEYRAATLLIKTEFPDGAASITKIEVDGDVISLDGHDVPLQTQSET
DTHGRISVRIRRSISSAPDSTSSIGNAVITPRHSNLTAEHLLDGDPSFGYP
PASPRLSGCASSDAYSLOPTPRASNFNETEVTAGTPVWGRSPVGGGRVS
RQMSPGKCQVEERQGC KDITQSDKEISFRDNIKVSMPGEEAADTTARNQ
KMPHAFVMIRLILTVVGRKLSRNPNTYSSVLGLVWVSLISFKWNMEMPSL
IKASIKIISDASLGMAMFSLGLFMALQPRIIACGTKRAAMGMAIRFVCGP
LVMSASSIVIGLRGDKLHTAIVQAALPQGI VPFVFAREYGLHPDILSTGVI
FGMLVSLPITLLYYILLGL-

B

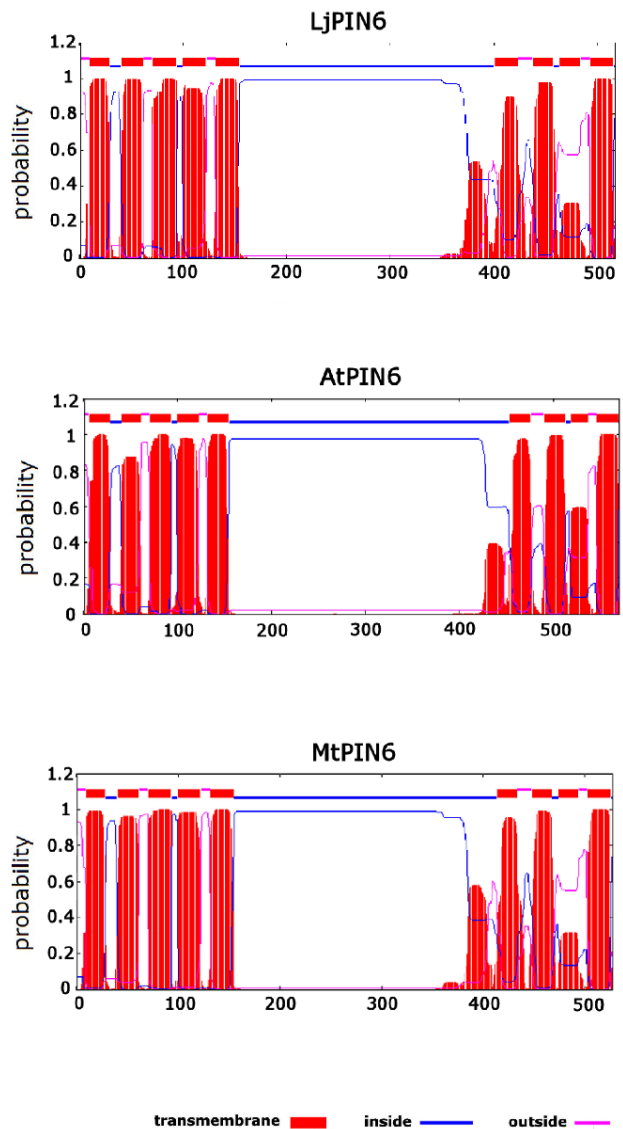


Figure S5. Complete sequence of LjPIN6 and its transmembrane domain topology. (A) LjPIN6 coding and protein sequence with the fragment obtained through the sequencing (bolded) and binding sites of primers used for sequencing (shaded). (B) Transmembrane domain topology of LjPIN6 and its orthologs from *A. thaliana* and *M. truncatula*. Red peaks represent transmembrane helices.

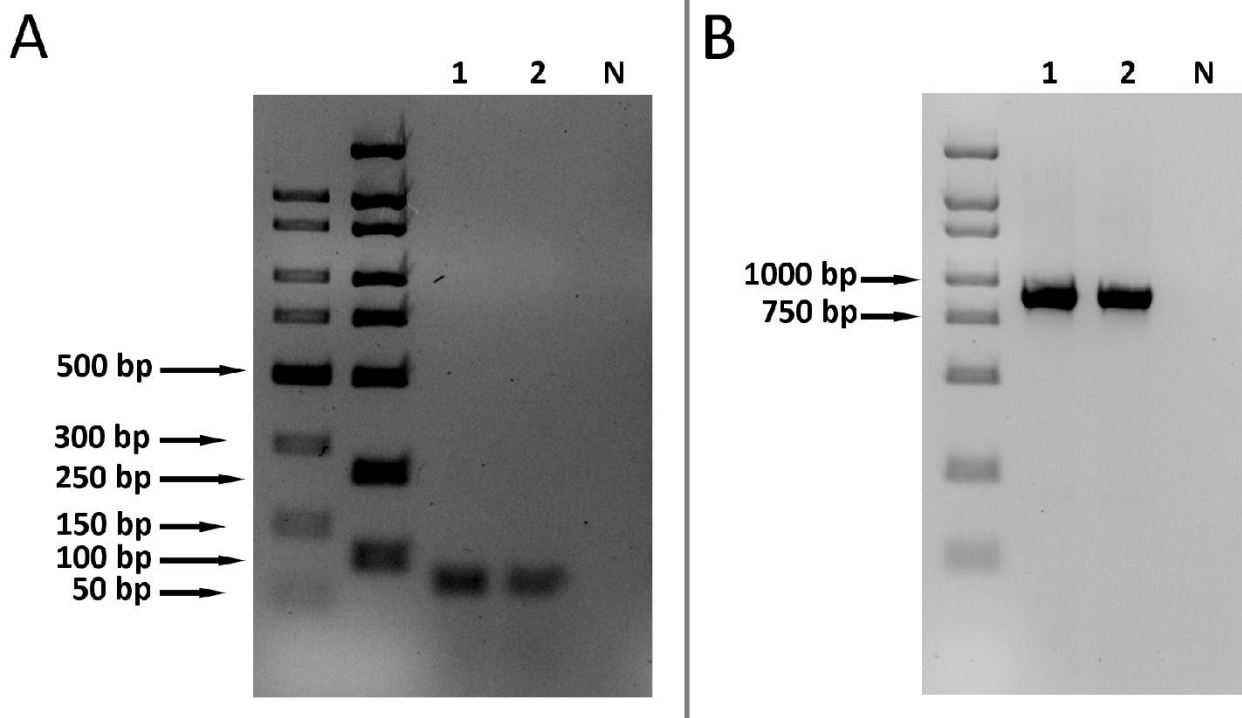


Figure S6. The electrophoretic separation of *LjPIN5* (A) and *LjPIN8* (B) products of amplification performed on genomic *L. japonicus* DNA template. Wells number 1 and 2 represent two biological repetitions, wells “N” state for negative controls (PCR performed in the same conditions as other samples, with the same volume of ingredients, but without DNA template). bp- base pairs.

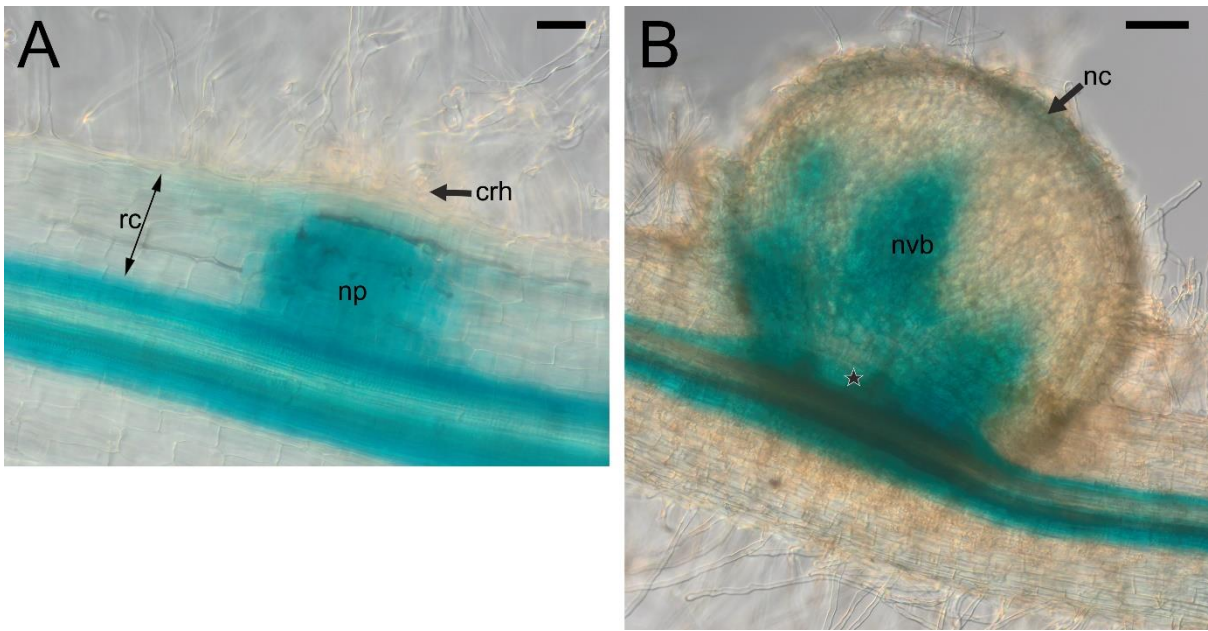


Figure S7. The pattern of *LjPIN1* expression in the "hidden" (A) and "emerged" (B) root nodule primordia. Labels: crh – curled root hair; nc – nodule cortex; np – nodule primordium; nvb – nodule vascular bundle; rc – root primary cortex; asterisk – vascular connection with the root stele within the nodule base. Bars: 50 μm (A), 100 μm (B).

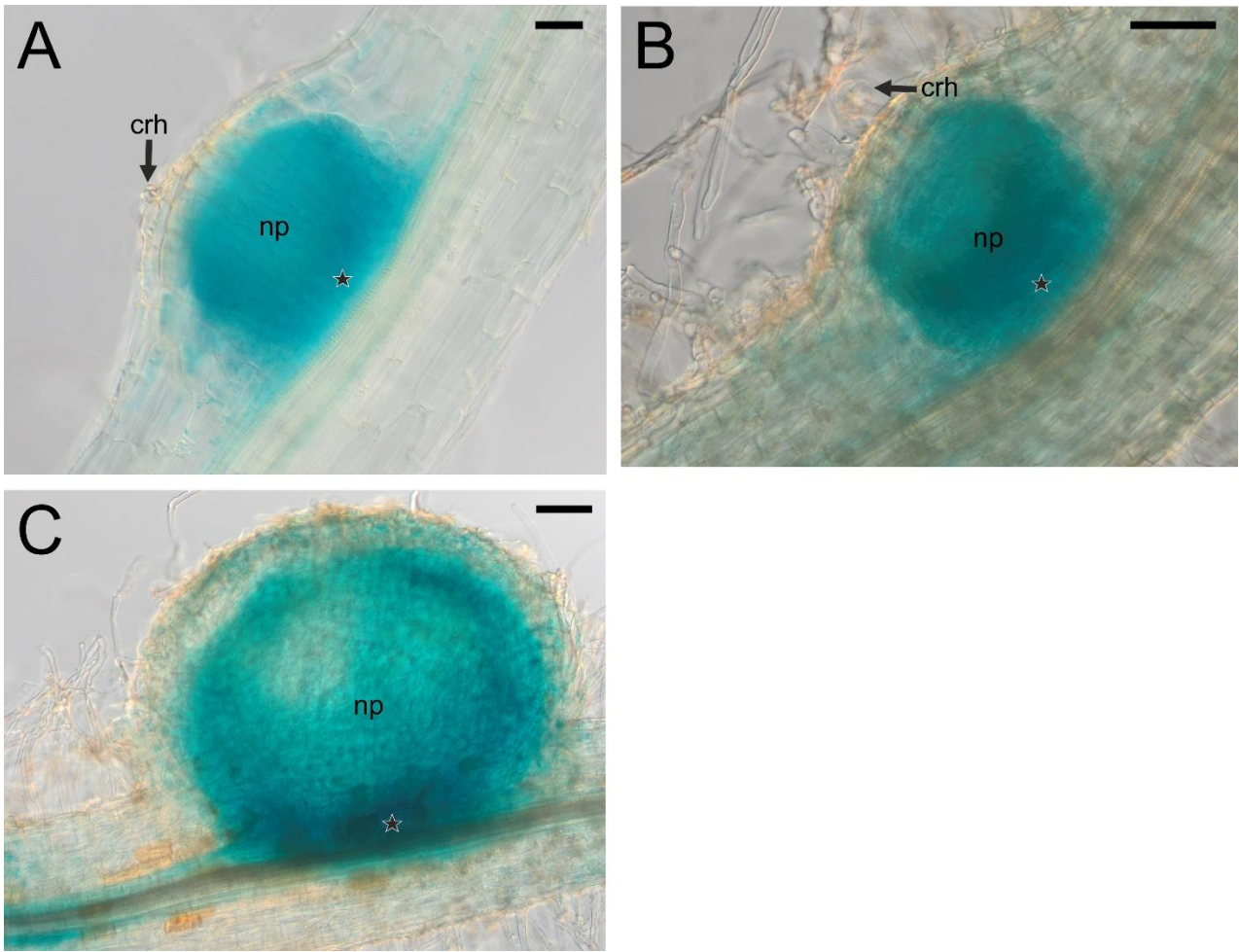


Figure S8. The pattern of *LjPIN2* expression in the "hidden" (A, B) and "emerged" (C) root nodule primordia. Labels: crh – curled root hair; np – nodule primordium; nvb – nodule vascular bundle; asterisk – vascular connection with the root stele within the nodule base. Bars: 50 μm (A), 100 μm (B, C).

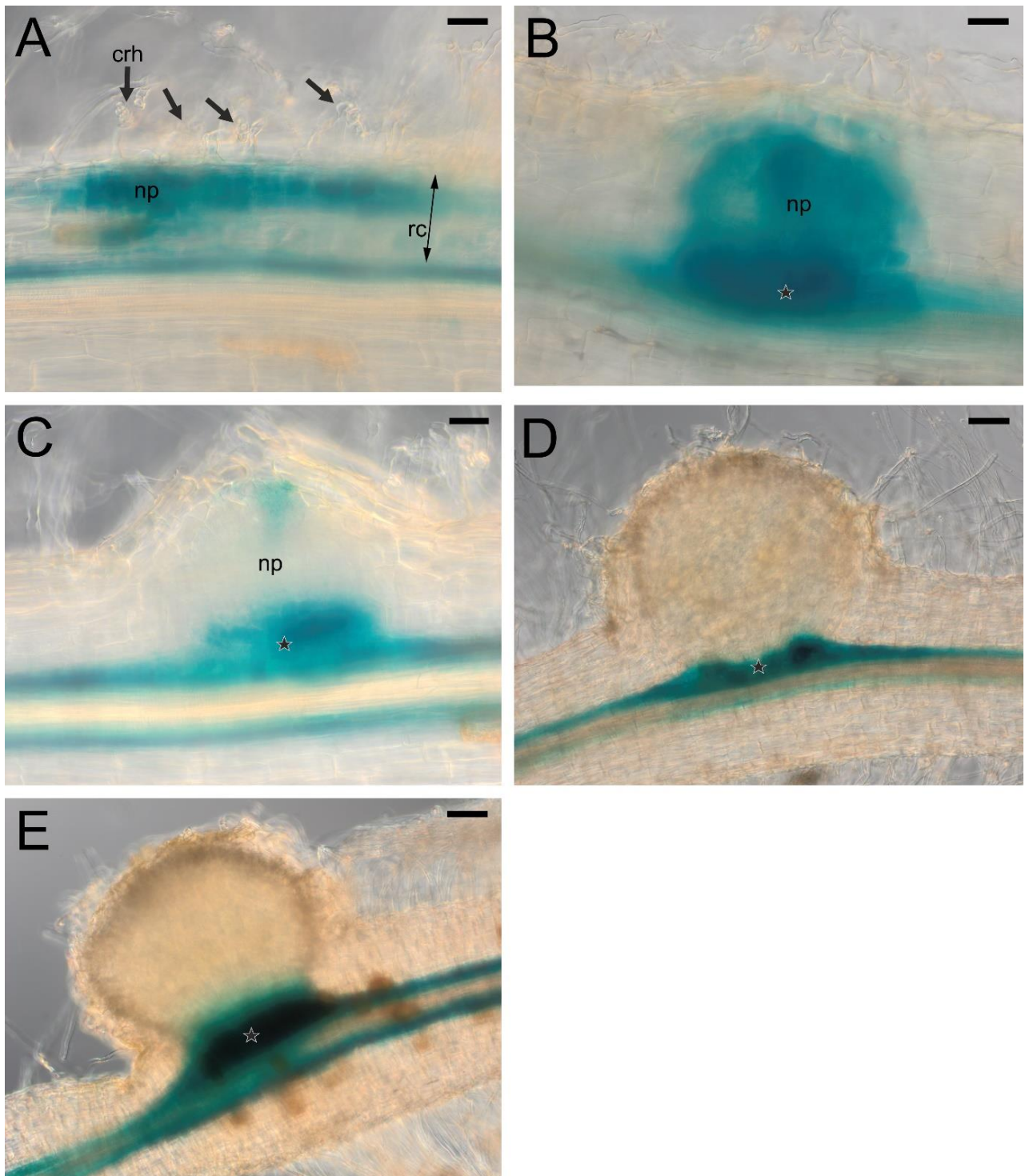


Figure S9. The pattern of *LjPIN3* expression in the initial (A), "hidden" (B), "emerging" (C) and "emerged" (D) root nodule primordia and in juvenile, 20 dpi, nodules (E). Labels: crh – curled root hair; np – nodule primordium; rc – root primary cortex; asterisk – vascular connection with the root stele within the nodule base. Bars: 50 μ m (A-C), 100 μ m (D, E).

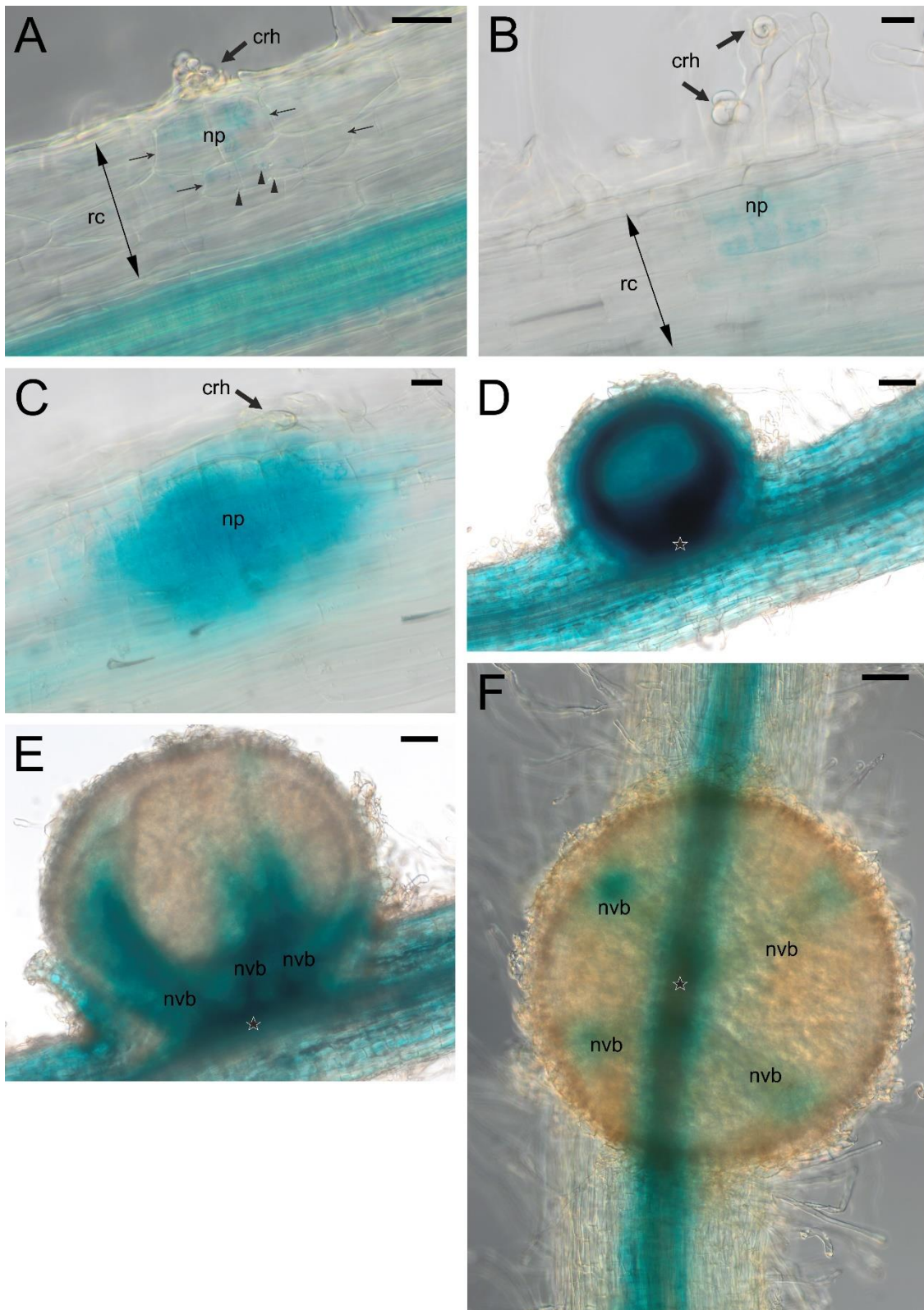


Figure S10. The pattern of *LjPIN4* expression in the initial (A, B, C) and “emerged” (D) root nodule primordia and in juvenile, 20 dpi, nodules (E, F).

Labels: crh – curled root hair; np – nodule primordium; nvb – nodule vascular bundle; rc – root primary cortex; thin black arrows – divided cells of root cortex constituting initial primordium of the nodule; arrowheads – starch grains; asterisk – vascular connection with the root stele within the nodule base. Bars: 20 μm (B, C), 50 μm (A), 100 μm (D-F).

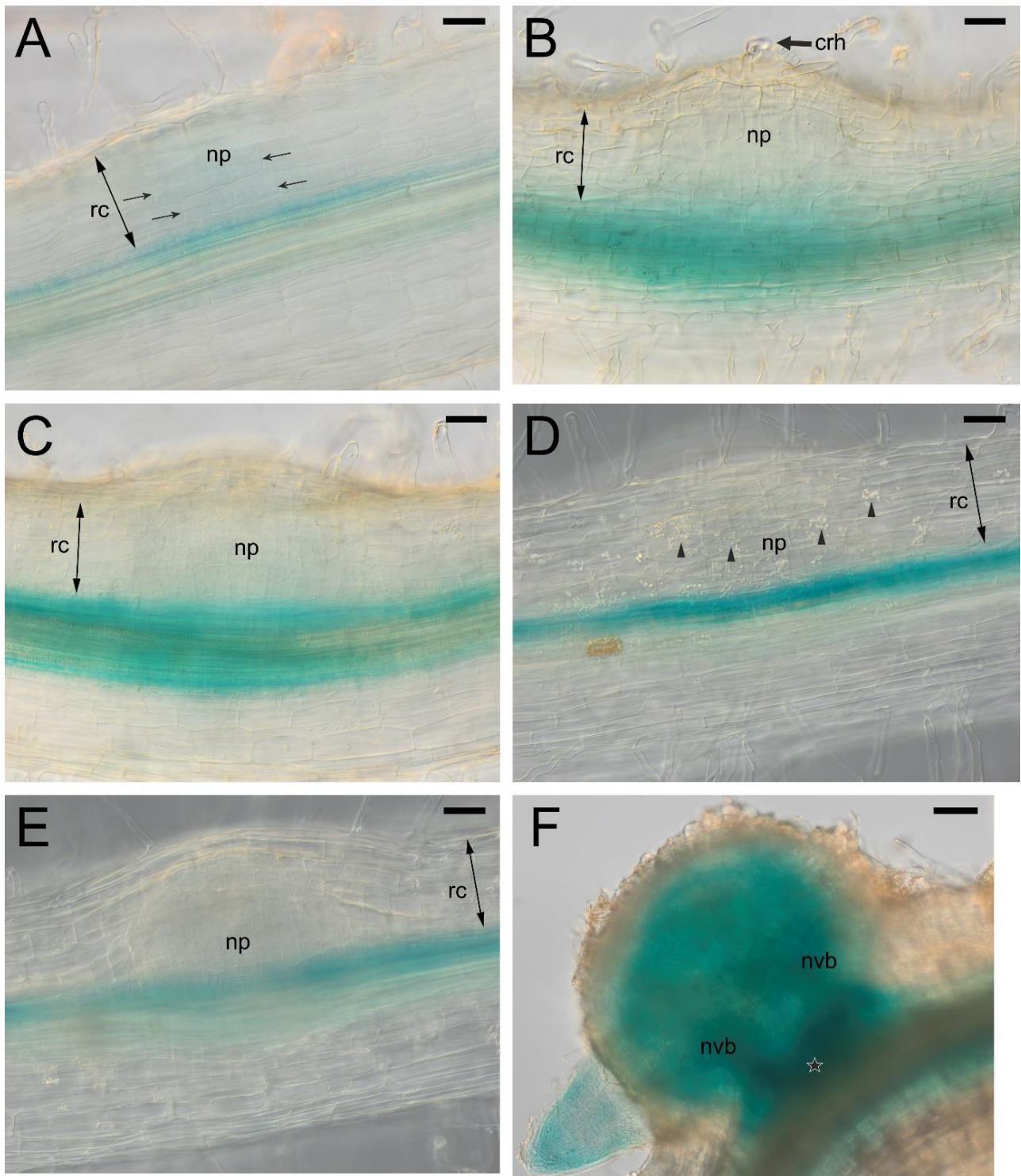


Figure S11. The pattern of *LjPIN5* expression in the initial (A), “hidden” (B, C, D, E) and “emerged” (F) root nodule primordia.

Labels: crh – curled root hair; np – nodule primordium; nvb – nodule vascular bundle; rc – root primary cortex; thin black arrows – divided cells of root cortex constituting initial primordium of the nodule; arrowheads – starch grains; asterisk – vascular connection with the root stele within the nodule base. Bars: 50 μm (A-C), 100 μm (D-F).

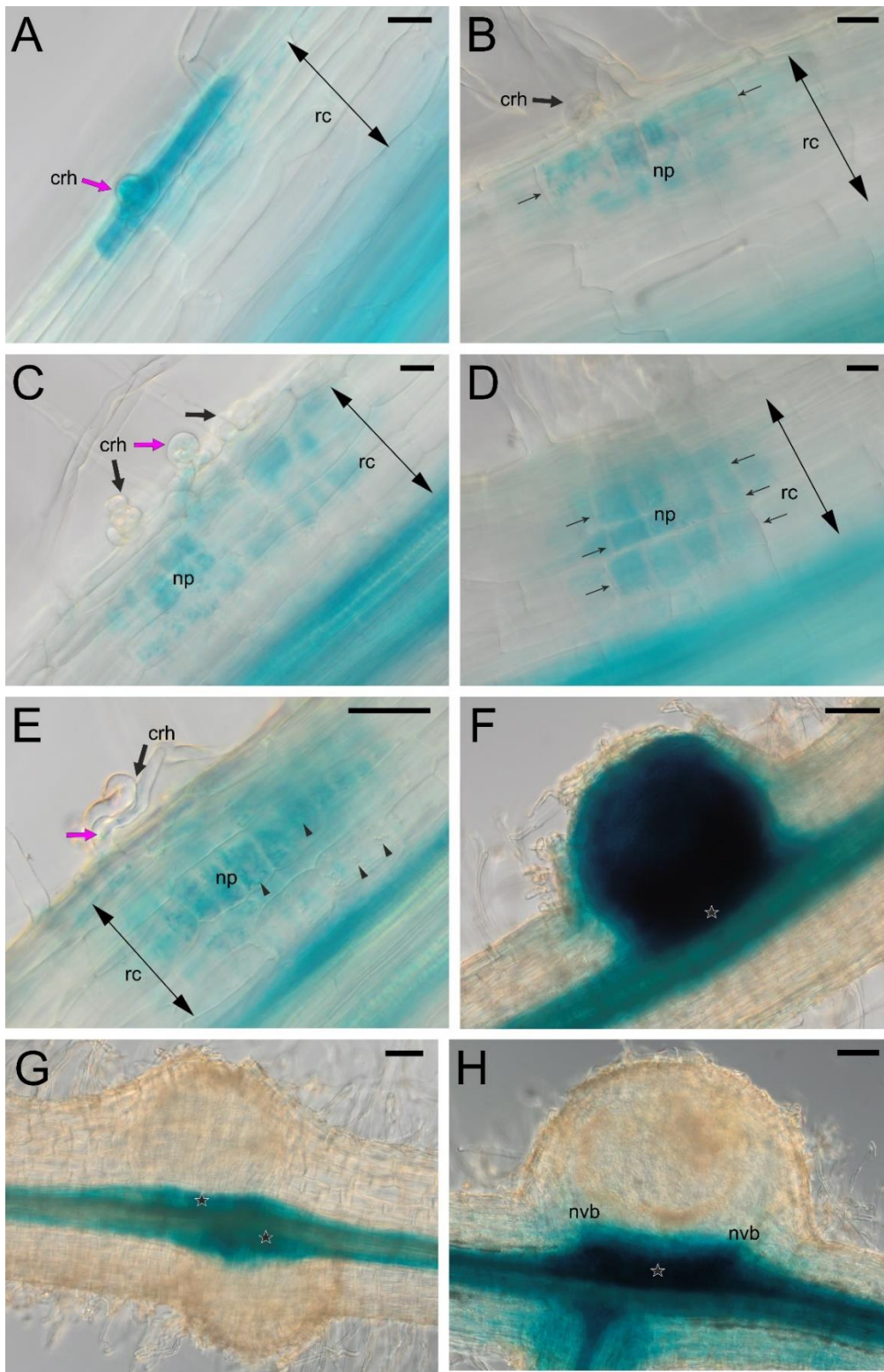


Figure S12. The pattern of *LjPIN6* expression in the initial (A, B, C, D, E) and “emerged” (F, G) root nodule primordia and in juvenile, 20 dpi, nodules (H).

Labels: crh – curled root hair; np – nodule primordium; nvb – nodule vascular bundle; rc – root primary cortex; pink arrow – curled root hair expressing GUS signal; thin black arrows – divided cells of root cortex constituting initial primordium of the nodule; arrowheads – starch grains; asterisk – vascular connection with the root stele within the nodule base. Bars: 20 μ m (A-D), 50 μ m (E), 100 μ m (F-H).

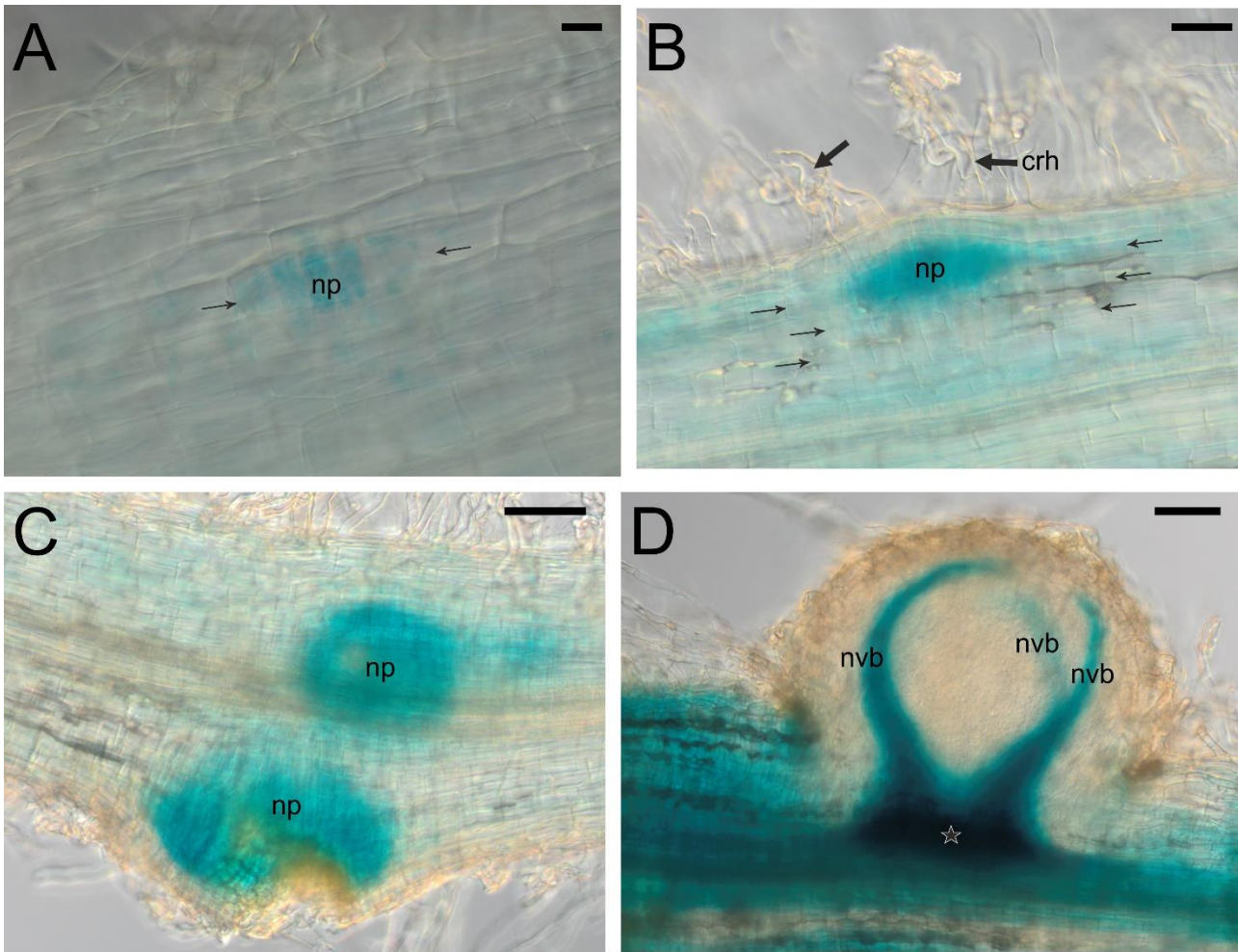


Figure S13. The pattern of *LjPIN7* expression in the initial (A, B), “hidden” (C) and “emerged” (D) root nodule primordia and in juvenile, 20 dpi, nodules (E).

Labels: crh – curled root hair; np – nodule primordium; nvb – nodule vascular bundle; thin black arrows – divided cells of root cortex constituting initial primordium of the nodule; asterisk – vascular connection with the root stele within the nodule base. Bars: 20 μm (A), 50 μm (B), 100 μm (C, D).

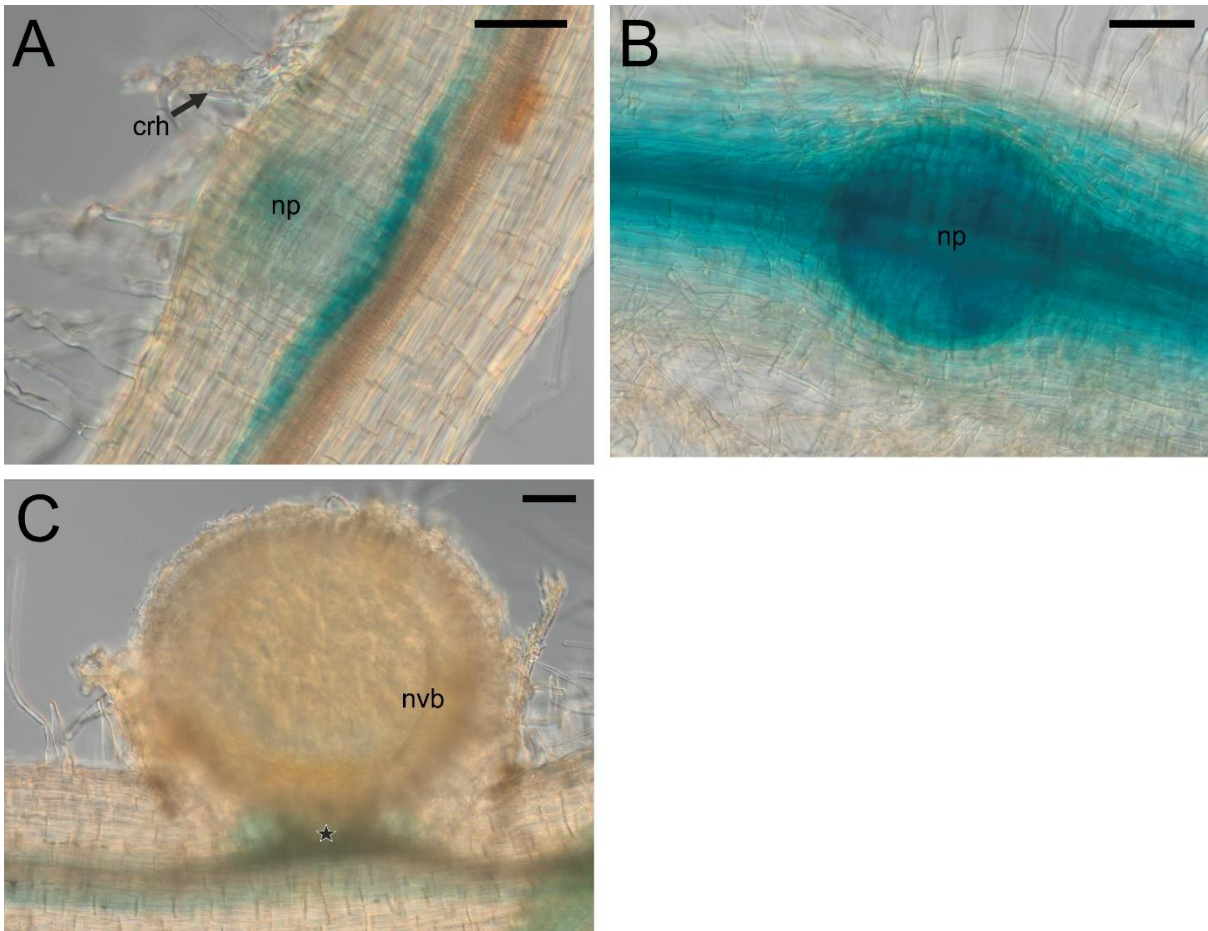


Figure S14. The pattern of *LjPIN8* expression in the initial (A) and "hidden" (B) root nodule primordia and in juvenile, 20 dpi, nodules (C).

Labels: crh – curled root hair; np – nodule primordium; nvb – nodule vascular bundle; asterisk – vascular connection with the root stele within the nodule base. Bars: =100 μm.

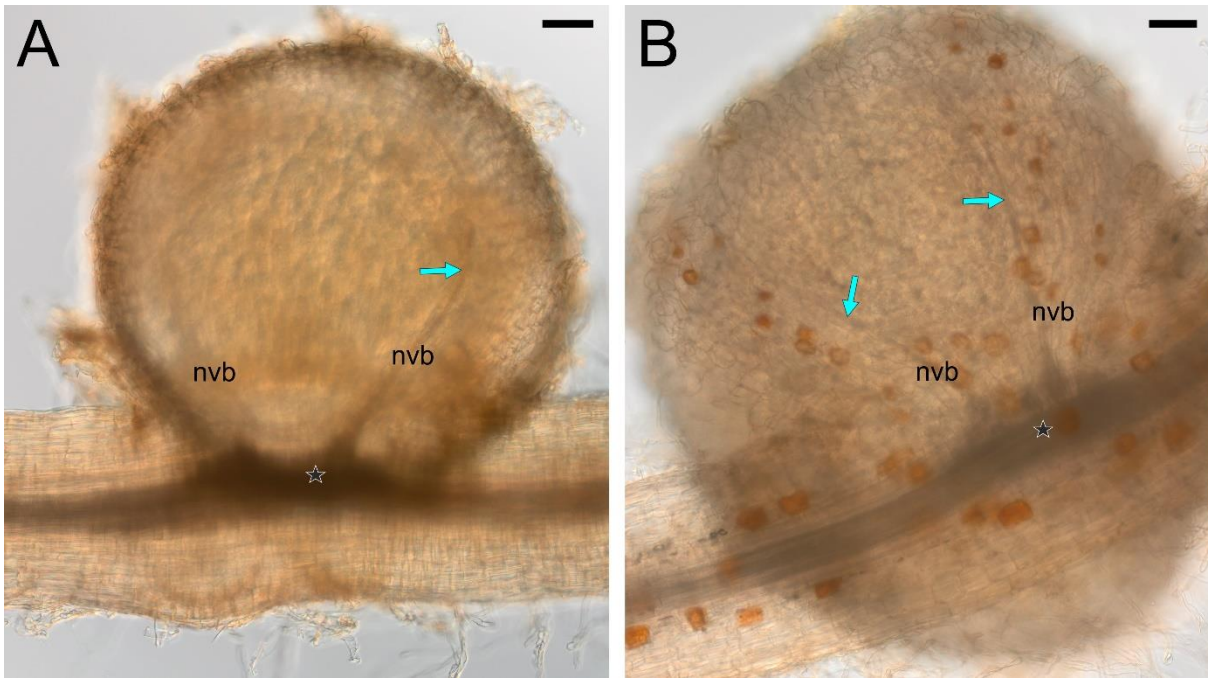


Figure S15. The control *L. japonicus* nodules collected from plants with adventitious roots formed after cutting off the root system (as in regular transformation process) but not transformed with *A. rhizogenes* (A), and plants transformed with non-transgenic *A. rhizogenes* (B).

Labels: nvb – nodule vascular bundle; blue arrows – files of tracheary elements in the vascular bundle; asterisk – vascular connection with the root stele within the nodule base. Bars: 100 μ m.

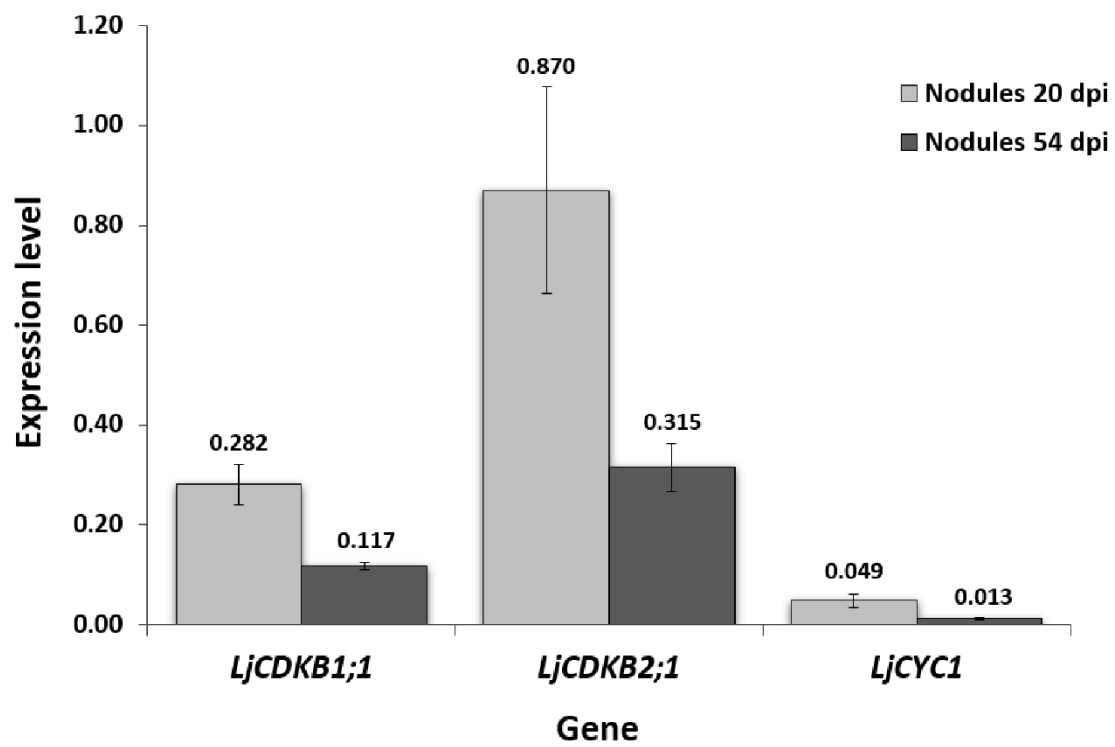


Figure S16. Normalized expression level of G2-M phase cell cycle markers. Mean values (\pm SE) are derived from three biological replicates, for which three individual qPCR reactions were performed.

Table S1. RT-PCR conditions.

Temperature	Time
98 °C	30 s
35 cycles:	
98 °C	10 s
59.5 °C	30 s
72 °C	1 min
72 °C	10 min

Table S2. Real-time qPCR conditions.

Temperature	Time
PCR	
50 °C	20 s
95 °C	10 min
40 cycles:	
95 °C	15 s
60 °C	1 min
Melting curve	
95 °C	15 s
60 °C	1 min
95 °C	30 s
60 °C	15 s

Table S3. Accessions of genes, sequences of primers and product lengths for real-time qPCR. bp - base pairs.

Gene / ID	Forward primer sequence 5' → 3'	Reverse primer sequence 5' → 3'	Product length [bp]
<i>LjPIN1</i> (Lj4g3v3114900.1)	ATACTCTCTACAATCCTCAAGGAACC	GTTCCACCAGAAATAGCATCATAGT	150
<i>LjPIN2</i> (Lj4g3v2139970.1)	CCCACTATTGTAAAAGGTTCCATC	AGCCATAGAAAATGTTGCTACAGA	138
<i>LjPIN3</i> (Lj0g3v0320849.2)	ATCGCTATGTACGGCGATTACT	GGAGTCAACCTTGAATGACACAAT	174
<i>LjPIN4</i> (Lj4g3v0633470.1)	ATTTCTTACAGGTCCTGCAGTTATG	GGTAGAGCTATCAACATCCCAAATA	192
<i>LjPIN5</i> (Lj1g3v2809230.1)	TCGCCCTGTTTTAGGCTAC	TATCGCATCGCACTGTTCTC	71
<i>LjPIN6a</i> (Lj0g3v0178829.1)	CTCTATATTCGCAATGCTGGTAG	CTATGATGAACCTTAGCGTCCATCTC	174
<i>LjPIN6b</i> (Lj1g3v0264160.1)	GAAAACCTTCGCGTAACCCTAATA	CATGAAAAGCCCTAAGCTAAACAT	167
<i>LjPIN7</i> (Lj1g3v4106960.1)	GCTAGTGTGTCATGACTAGGCTCATTT	GCCTGCATCAGATAGAATTGATATT	171
<i>LjPIN8</i> (Lj2g3v1034600.1)	ATAACCGGTCTATCATGTGCAACAC	TGTAGCTCCTGAGAAGTCTCACTTT	242
<i>LjCDKB1;1</i> (Lj3g3v2061650.1)	CGATCTCAAGAAATACATCGATACC	TACAGAGCTGAAACAGAAAACCTCTGAA	95
<i>LjCDKB2;1</i> (Lj5g3v0279150.1)	AGGAACTTATGGGAAGGTGTATAGG	TGTTTAACATCCATTAACCTGACAA	180
<i>LjCYC1</i> (Lj2g3v1645400.1)	CATATGTTTTTCATGAGAAGGTTCTTA	CATACTCAACTAGAGACAGCTCAACC	99
<i>LjPP2AA1</i> (Lj2g3v1155670)	GCAGTGCTTATAGACGAGCTGA	TGGATAGCTTGCGAATTGAG	70
<i>LjPP2AA2</i> (Lj2g3v0742070)	TCGACGAGTTGAAGAACGAG	GTGTCCTCTCCTACCAAGC	93

Table S4. PCR conditions.

Temperature	Time
95 °C	5 min
40 cycles:	
95 °C	30 s
60 °C	30 s
72 °C	30 s
72 °C	5 min

Table S5. Sequences of primers for promoter amplification. Shaded parts of the sequence represent fragments for Gateway cloning, while underlined nucleotides in the primers for elongation PCR show complementarity to the template.

	Forward primer sequence 5' → 3'	Reverse primer sequence 5' → 3'	Position of promoters
<i>pLjPIN1</i>	AAAAAGCAGGCTCCTAAACCATTGTT CCGATCTTAC	AGAAAGCTGGGTCCTTCTCACTTCGGT AGAGAAACA	Chromosome 4 42168645..42170156
<i>pLjPIN2</i>	AAAAAGCAGGCTCCATAGGGAACCT CCTAGATCG	AGAAAGCTGGGTCGGCTAAACCAAG GTTGTTGT	Chromosome 4 (-) 29544633..29546630
<i>pLjPIN3</i> , PCRI	AAAAAGCAGGCTCCTCCCATGAGTCA AGTTATCC	GTACTCAAAGAGGAATAAGAGGAG	-
<i>pLjPIN3</i> , PCRII	AAAAAGCAGGCTCCTCCCATGAGTCA AGTTATCC	AGAAAGCTGGGTCCTTTTTTTTGTGG GTTTTA	Chromosome 0 (-) 167444230..167446018
<i>pLjPIN4</i>	AAAAAGCAGGCTCCAAGAATAAGCC TCTCGTTTCTCAA	AGAAAGCTGGGCTTTCTTTATATGGC CAAAAAAAG	Chromosome 4 10020316..10021906
<i>pLjPIN5</i> , PCRI	AAAAAGCAGGCTCCTATGATGATGT CCAAGCTGA	GAGAAGCAAAGTGAGCCATA	-
<i>pLjPIN5</i> , PCRII	AAAAAGCAGGCTCCTATGATGATGTC CAAGCTGA	AGAAAGCTGGGTCCTTTTTTAATTTT TTTCTTGCTCTCG	Chromosome 1 (-) 31944557..31946536
<i>pLjPIN6</i>	AATTCTAAGGTGGGGTGGTTAAAAA	CTTTGAGTGAGATAGAAAGTGGTGT	Chromosome 1 Unmapped region
<i>pLjPIN7</i>	AAAAAGCAGGCTCCAGAAATTTAAA ACCCACAATTCCA	AGAAAGCTGGGCTTGTTTTAGCAGA AACAATCACT	Chromosome 1 48774196..48776085
<i>pLjPIN8</i>	AAAAAGCAGGCTCCTTCATACAGAAT CACCACGA	AGAAAGCTGGGTCGATTCCTAAGTAT TGAAAGA	Chromosome 2 (-) 16542704..16544503
Elongation PCR <i>pLjPIN6</i>	GGGGACAAGTTTGTACAAAAAAGCA GGCTCCAATTCTAAGGTGG	GGGACCACTTTGTACAAGAAAGCTG GGTCTTTTGAGTGAGATA	-
Elongation PCR <i>pLjPIN1-5</i> , <i>pLjPIN7-8</i>	GGGGACAAGTTTGTACAAAAAAGCA <u>GGCT</u>	GGGACCACTTTGTACAAGAAAGCTG <u>GGT</u>	-

Table S6. Conditions of promoter regions amplification.

Temperature	Time
98 °C	5 min
35 cycles:	
98 °C	30 s
58 °C – <i>pLjPIN6</i>	
60 °C – <i>pLjPIN</i> : 2, 3, 8 (PCRI, PCRII), 5 (PCRII)	30 s
66 °C – <i>pLjPIN</i> : 1, 4, 7	
70 °C – <i>pLjPIN5</i> (PCRI)	
72 °C	1 min
72 °C	5 min

Table S7. Conditions of promoter regions elongation with *att* sequences.

Temperature	Time
98 °C	30 s
30 cycles:	
98 °C	10 s
60 °C	30 s
72 °C	1 min
72 °C	5 min