

Supplementary Material

Overexpression of a soybean 4-coumaric acid: coenzyme A ligase (*GmPI4L*) enhances resistance to *Phytophthora sojae* in soybean

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1      GGGAGAAGAAAAACA AAAACATGATTTTTTGGCGCTTGGTCCGCAATGTATCGATGATGTT
61     GTGTCCCCATCACAAAGTTGATTGTCAGATAAAAAACATTAAATTCATCCAACATTGGGAA
121    GAAGAAATAAAGTGAAGCAGCAAAAGAAAGCGAGTAATCTTTCCGTGAGATCGGTGTGTGAA
181    TTGTATATTCCCATTTCAATGCGAGATCCCGTTATGGTCCGACGGAAATATACAGGTCA
      MQRSGYGS DGIYRS
241    CTCAGACCTTCCATCGTTTTTCCAAAAACTCAAACCTCTCACTCGTTTCCCATCTTTTC
      LRPSSI VFPKNS NLSL VSHLF
301    AACAGAGTCGACAGCCTTCCATCAAAACCAGCTCTTATCGATGCAAGACTTTCAGAAAACA
      NRVA AAFP SKPAL IDADS SET
361    CTTTCTTTCCGCGGAAC TCAAAATTGCTAAGTGTAGGGTTCGCTCACGGCCTCCTTCGTCTC
      LSFA ELKLT V RVA HGLRL
421    GGAGTGACCAAAAAACGACGTCGTTCTCTTCTCGCTCCCAACGACATCCGCTACATCGTC
      GVTKN D VV LFL APND IRYI V
481    TGCTTCTCGCCGTCGCTCTCTCGGCGCTGCGCTCACCACCGTCAACCCCGCCTACACC
      CFLAVASLGA AVT TVN P AYT
541    GCCCGCGAAGTCTCAAACAGGCCAACGACTCCAAACCCAAACTTTTGGTAACCGTCCGCC
      AA E V S K Q A N D S K P K L L V T V A
601    GAATTGTGGGACAAACTCGAACACCTCAAACCTCCCGCGGCTCTTCTACGTTGTTCCAAC
      ELWD KLEHLKLP AVFL RCSN
661    GCTCCCCACGCGCCATCCAGCGCGACTAGCTTCGACGCGCTGGTCAACTCGCCGGGTGCG
      APHAPS S A T S F D A L V Q L A G S
721    GTGACCGAGTTTCCCGAAATTA AAATTAACAGAGTGACACTGCGGGCAC TGTGTATTCT
      VTEFP FEIKI KQS D T A A L L Y S
781    TCGGGTACCACCGGCTTGAGCAAGGGCGTGGTCTCACGCGACGGGA ACTTCGTCGCGGCC
      SGT T G L S K G V V L T H G N F V A A
841    TCGTTGATGATTGCTCGACGATGATTTGOCAGGGGTGTTGOCATAGCGTGTTCCTCTOC
      SLMIG FDDDL AGV LHS VFLC
901    GTTTTGCCTA TGTTCATGTTTTGGTTTGATGGTCATTTCGTACGGACAGCTTCAGAGG
      VLP M F H V F G L M V I S Y G Q L Q R
961    GGTAGTCCGCTGTGTCTCAAGAAAGTTGAGTTGAAATGOTTCTGAAAGACCATTGAG
      GSA V V S L K K F E F E L V L K T I E
1021   AAGTTTAAAGGTGACGCAATTTGCGGTTGTCCTCCGATTATTCTTCTCGCGAAACAC
      KFKV THLV VVPPI ILALAKH
1081   GGTTTGGTTGATAAGTATGATCTTTCGCTCTCAAGCATATTGTTCTGGCGCTGCTCCT
      GLVD K Y D L S S L K H I G S G A A P
1141   CTTGGGAAAGAGTTGATGAAGGAGTGTCTAAACGTTTTCTCATGCCATTGTTTCTCAG
      L G K E L M K E C A K R F P H A I V S Q
1201   GGATATGATGACTGAAACTGTGGAATGTTTCTGTGGAGAAATGCAAGGATGGGAATT
      G Y G M T E T C G I V S V E N A R M G I
1261   CGGAATAGTGGCTTACGGGAATGCTGTGCGCCGGGAATGGAGGCTCAAGTAGTTAGTGTG
      R N S G S T G M L V A G M E A Q V V S V
1321   GATACTCTGAAGCCTCTCCCTCCGGAACAGTTGGGGGAGATATGGGTACGGGGCCCTAAT
      D T L K P L P P Q L G E I W V R G P N
1381   ATGATGCAAGGTTATCAACAATCCACAAGCCACAAGATTGACTATGGATAAAAAGG
      M M Q G G Y H N N P Q A T R L T M D K K G
1441   TGGGTACATACAGGAGATCTGGATATTTGATGAGGATGGGCAACTTTTTGTGTGTTGAC
      WVHT GD LG Y F D E D G Q L F V V D
1501   CGAATCAAAGAACTGATCAAGTATAAAGGTTTTCAGGTTGCGCCGGCAGAACTTGAAGGG
      R I K E L I K Y K G F Q V A P A E L E G
1561   CTTCTGGTTTCTCATGCTGAAATACTIGATGCTGTGTTGTCATCCCATATCCTGATGCTGAA
      LLVSH AEILCD AVVIP YPD AE
1621   GCTGGTGAGGTTCCAGTTGCTATGCTTTCGTTTACCCCAATAGTTCACTCACAGAGGAA
      AGEVPVA YVV VRS P N S S L T E E
1681   GATGTTCAAAAATTTATGCTAAGCAGGTAGCGCCTTCAAAAAGAATAAGAAGAGTGACA
      DVQK FIAKQVAP FKRIRRV T
1741   TTCATCAATGCTGTTCTAAGACAGCATCAGGAAAAAATTCTCCGAAGAGAGCTTATTGAG
      F I N A V P K T A S G K I L R R E L I E
1801   AAAGTAAGATCCAAAATATGATGATATGGTGTAAATTTTTTGTGTGTTTTCAAACC
      K V R S K I
1861   ATTTCGGGGTTTAAATTCAGAAAGTAGAAATGCAAGTCCAGAGCGAATCCGGAAAAGGTATGA
1921   TGGTTGTCATAGTGGATCAAAATATGTAICTGGCTGGGAACAGTACTACTGTTGGGTCTA
1981   GCATATTTCAATCCATTTAATAATGGTCAATTCATTCTAGTTCACTTAAGGTTGAATC
2041   TTTAAAAAAATGGTCTGATGTGACCTACCATGGATTGGTGAGATAAACAGGTTGACCCAT
2101   CAATTTACCTAGAATATTTTTTACTTAAAAAAAATCAACAAAAAAGTTAATTTTT

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Fig. S1. Sequence of *GmPI4l* gene. Nucleotide sequences of *GmPI4l* cDNA and the encoded amino acid sequence.

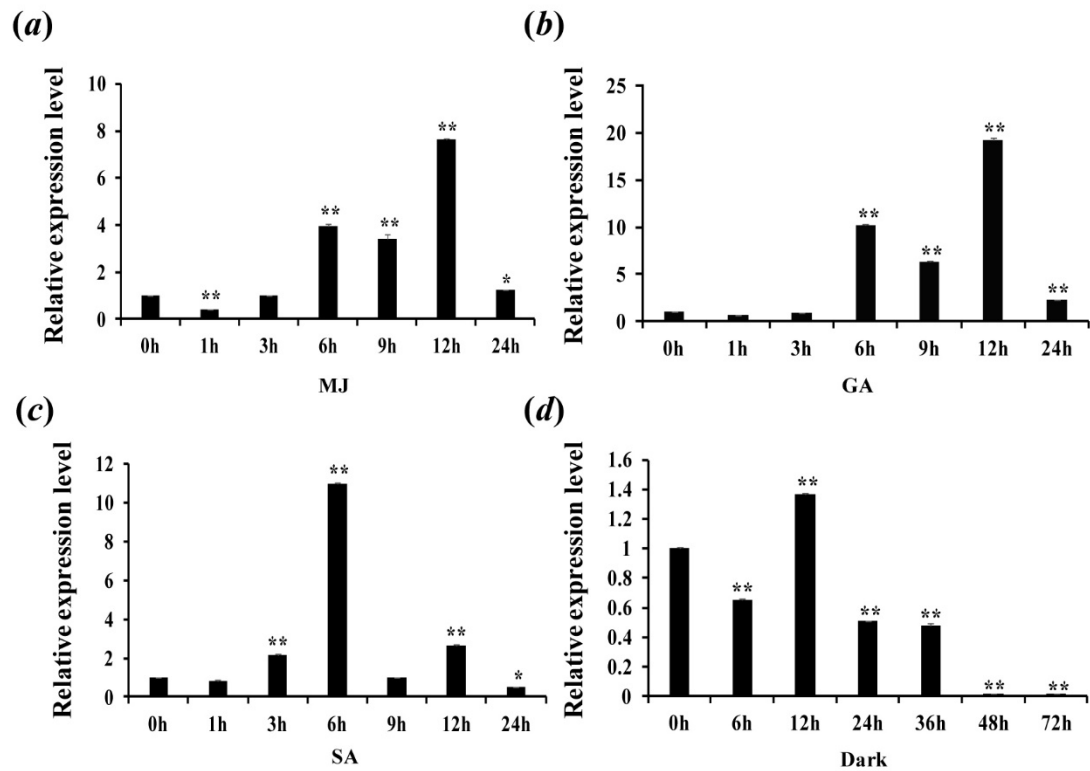


Fig. S2. The seedlings were exposed to various treatments, including dark treatment, salicylic acid (SA), gibberellic (GA), and methyl jasmonate (MeJA). The seedlings were exposed to dark chambers for 0, 6, 12, 24, 36, 48 and 72 h. The seedlings were sprayed with 0.2 mM SA, 250 mg L⁻¹ GA and 100 μM MeJA, respectively. The leaves were sampled at 0, 1, 3, 6, 9, 12 and 24 h after the imposition of the treatments. The control groups (mock-treated plants) were treated with the same dilutions without phytohormone. Forty-two seedlings were used in each treatment (treatment and control groups). Three biological replicates with three respective technical replicates were performed and data were statistically analyzed using Student's t-test (*P<0.05, **P<0.01). Bars indicate standard error of the mean. The expression patterns of *GmPI4l* under hormone treatments were determined according to the method of Dong *et al* (2015).

Fig. S3. Southern-blot assay of the T4 *GmPI4l*-overexpress and non-transgenic soybean plants. Twenty micrograms of genomic DNA digested by the restriction enzyme Hind III was hybridized with the probe derived from the bar gene.

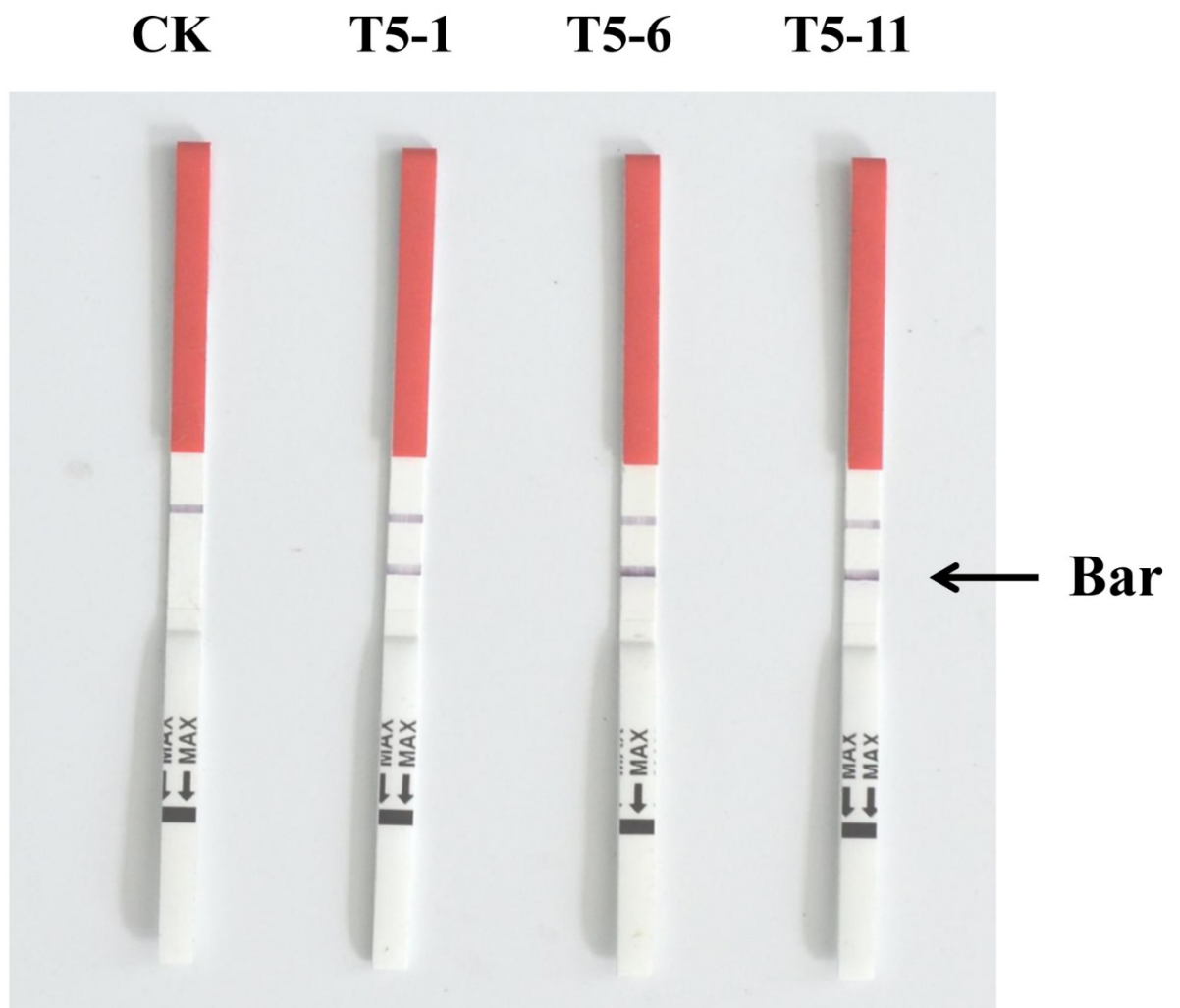


Fig. S4. Transgenic soybean plants were tested using Liberty Link strips.

Table S1. Oligonucleotide primers used in this study

| | | |
|---------------------|----------------------|-----------------------------------|
| Gene cloning | <i>Gm4CL-likeF</i> | CCCATGGCAATGCAGAGATCCGGTTAT |
| | <i>Gm4CL-likeR</i> | CCACGTGCTATTTTGGATCTTACTTTCTCAAT |
| | <i>Gm4CL-like-oF</i> | GGCCATGGATGCAGAGATCCGGTTATGG |
| | <i>Gm4CL-like-oR</i> | GGCACGTGCTATTTTGGATCTTACTTTCTCAAT |
| qRT-PCR | <i>Gm4CL-like-qF</i> | TGTTCCCAACGCTCCCA |
| | <i>Gm4CL-like-qR</i> | CACCCCTGCCAAATCATCG |
| | <i>GmActin4F</i> | GTGTCAGCCATACTGTCCCCATT |
| | <i>GmActin4R</i> | GTTTCAAGCTCTTGCTCGTAATCA |
| | <i>GmEF1F</i> | CCACTGCTGAAGAAGATGATGATG |
| | <i>GmEF1R</i> | AAGGACAGAAGACTTGCCACTC |
| | <i>GmTEF1F</i> | TGATCGTGCTGAACCACCC |
| | <i>GmTEF1R</i> | CGAGCGACGGTCCATCTT |
| GFP | <i>Gm4CL-like-GF</i> | CCCATGGCAATGCAGAGATCCGGTTATGG |
| | <i>Gm4CL-like-GR</i> | CACTAGTCTATTTTGGATCTTACTTTCTCAAT |