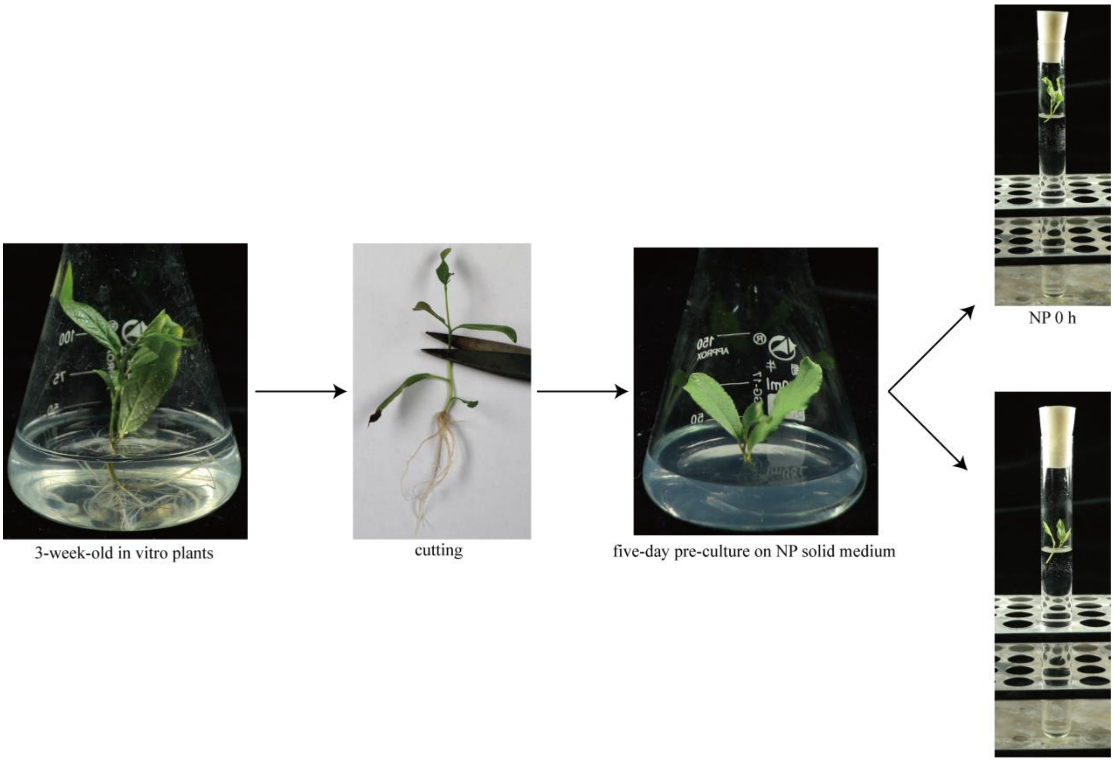
Supporting Information

The following Supporting Information is available for this article:

Figure S1 A scheme showing the experimental steps of low phosphate treatment experimental steps. LP, low phosphorus; NP, normal phosphorus.
Figure S2 Comparison of root length in WT treated with low phosphorus (LP) and normal phosphorus (NP) conditions.
Figure S3 Comparison of AR number in cuttings directly treated with low phosphorus (LP) and normal phosphorus (NP) conditions.
Figure S4 RT-qPCR analysis of Pi starvation induction related DEGs from RNA-seq experiment.
Figure S5 The multilayered hierarchical gene regulatory network (ML-hGRN) built with Bottom-up GGM algorithm where PuMYB40 and PuWRKY75 are located at the third layer and regulate LPR1 and ERF003. The genes involved in adventitious rooting (AR) related biological processes are located at the bottom layer.
Figure S6 PCR and RT-qPCR verified the expression level of *PuMYB40* in *PuMYB40*-overexpression (OE) and *PuMYB40-SRDX* lines.
Figure S7 Phylogenetic analysis and expression pattern of *PuWRKY75.*
Figure S8 PCR and RT-qPCR verified the expression level of *PuWRKY75* in *PuWRKY75*-overexpression (OE) and *PuWRKY75-SRDX* lines.
Figure S9 The promoter sequence of *PuLRP1* and *PuERF003*.
Figure S10 PCR and RT-qPCR verified the expression level of *PuLRP1* and *PuERF003* in *PuLRP1* and *PuERF003* transgenic lines, respectively.

Figure S1 A scheme showing the experimental steps of low phosphate treatment experimental steps. LP, low phosphorus; NP, normal phosphorus.



3-week-old in vitro plants

cutting

five-day pre-culture on NP solid medium

NP 0 h

LP 0 h

Figure S2 Comparison of root length in WT treated with low phosphorus (LP) and normal phosphorus (NP) conditions. Comparison of root length of *PuWRKY75* transgenic lines and WT under LP condition. values represent the mean ± SD of 50 plants. Significant differences compared based on one-way ANOVA and Duncan's multiple range test: **$p < 0.01$.

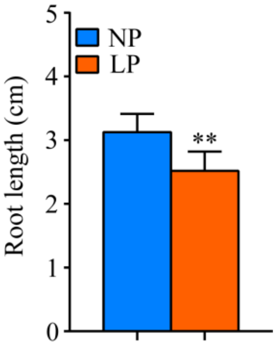


Figure S3 Comparison of AR number in cuttings directly treated with low phosphorus (LP) and normal phosphorus (NP) conditions. Each mean and standard deviation (SD) were calculated from 30 cuttings. The ns represents no significance of AR number in WT between LP and NP conditions, based on one-way ANOVA and Duncan's multiple range test.

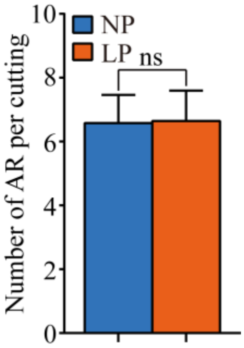


Figure S4 Quantitative analysis of the expression levels of Pi starvation induction related DEGs from RNA-seq experiment. *PuActin7* and *PuUBQ10* served as internal control. The asterisks indicate significant (** $p < 0.01$) difference between NP and LP stress, based on Student’s *t* test.

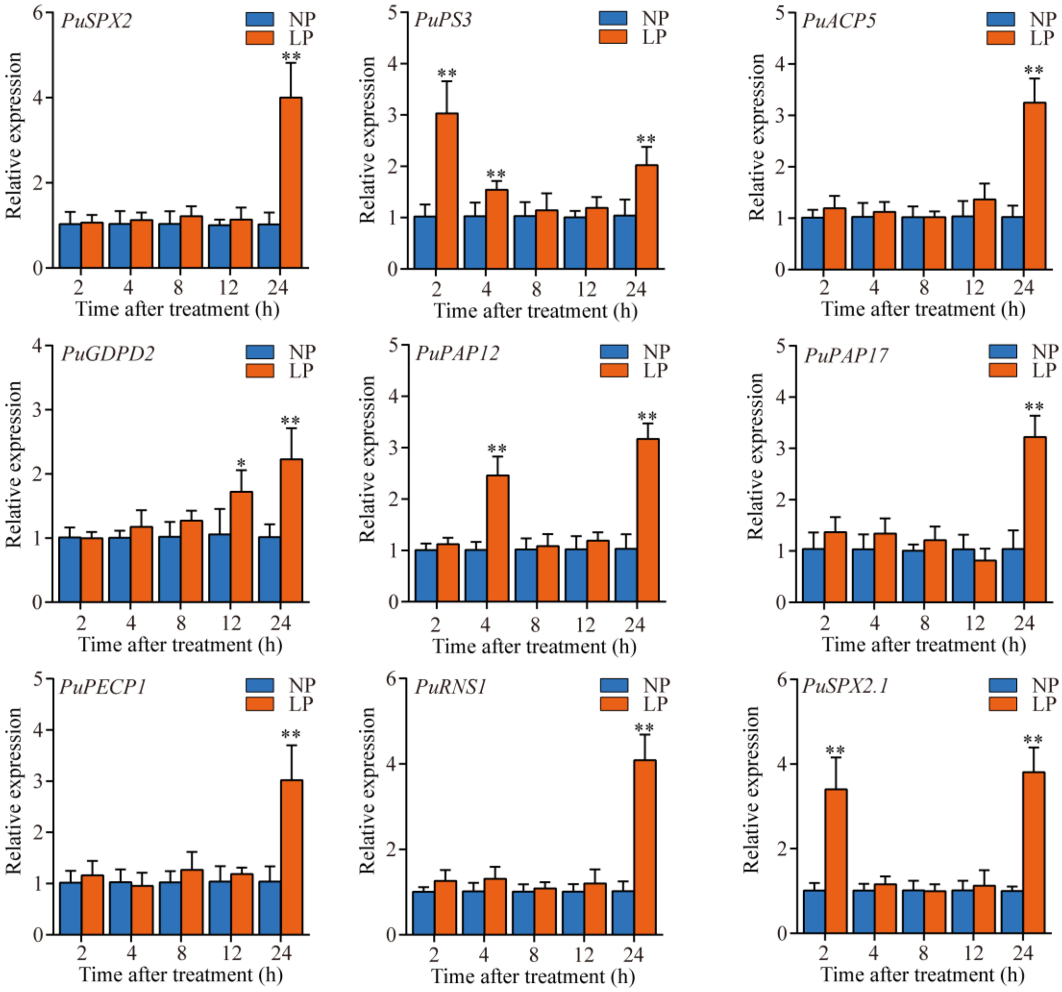


Figure S5 The multilayered hierarchical gene regulatory network (ML-hGRN) built with Bottom-up GGM algorithm where PuMYB40 and PuWRKY75 are located at the third layer and regulate LPR1 and ERF003. The genes involved in adventitious rooting (AR) related biological processes are located at the bottom layer.

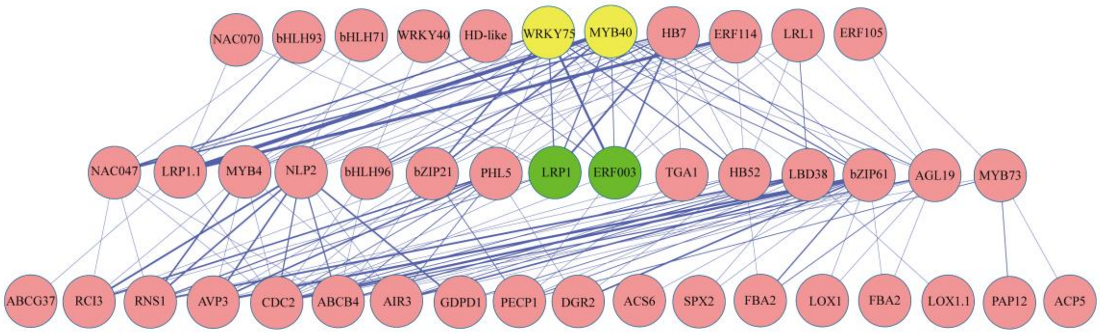


Figure S6 PCR and RT-qPCR verified the expression level of *PuMYB40* in *PuMYB40*-overexpression (OE) and *PuMYB40-SRDX* lines. (a) and (c) PCR confirmation of *PuMYB40-OE* and *PuMYB40-SRDX* lines, respectively. M represents DNA marker, P represents PCR product with pBI121-*PuMYB40* and *PuMYB40-SRDX* plasmid DNA as template, #1-13: PCR products with genomic DNA from resistant seedlings.

(b) and (d) Quantitative analysis of the expression levels of *PuMYB40-OE* and *PuMYB40-SRDX* lines, respectively. *PuActin7* and *PuUBQ10* served as internal control. The asterisks indicate very significant (** $p < 0.01$) difference between *PuMYB40* transgenic lines and WT, based on Student's *t* test.

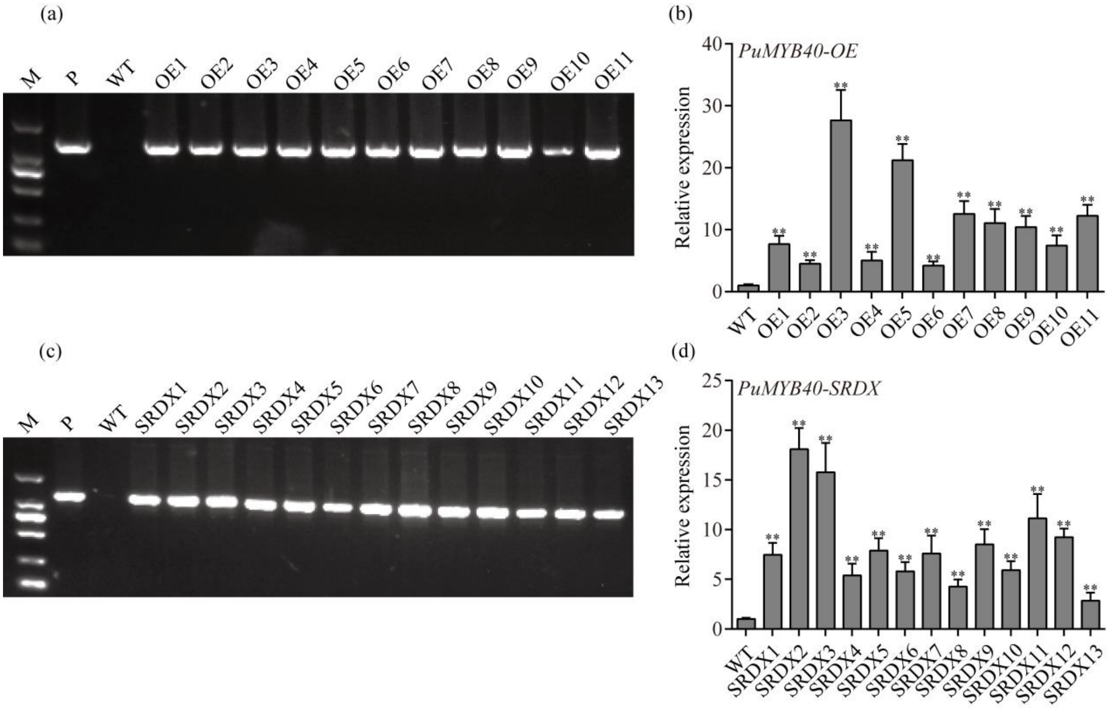


Figure S7 Phylogenetic analysis and expression pattern of *PuWRKY75*.

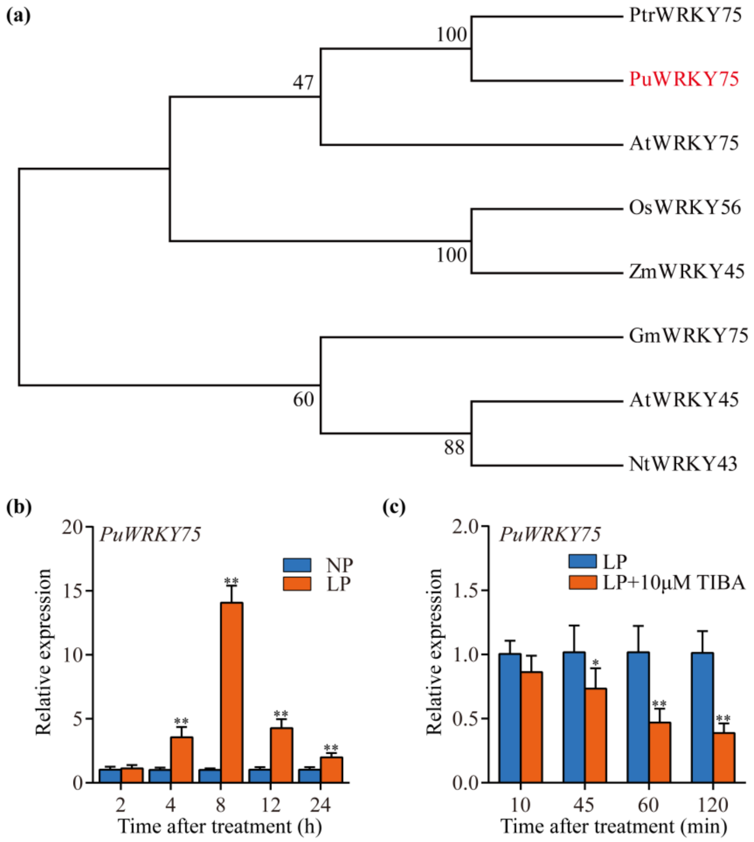


Figure S8 PCR and RT-qPCR verified the expression level of *PuWRKY75* in *PuWRKY75*-overexpression (OE) and *PuWRKY75-SRDX* lines. (a) and (c) PCR confirmation of *PuWRKY75-OE* and *PuWRKY75-SRDX* lines, respectively. M represents DNA marker, P represents PCR product with pBI121-*PuWRKY75* and *PuWRKY75-SRDX* plasmid DNA as template, #1-13: PCR products with genomic DNA from transgenic lines. (b) and (d) Quantitative analysis of the expression levels of *PuWRKY75-OE* and *PuWRKY75-SRDX* lines, respectively. *PuActin7* and *PuUBQ10* served as internal control. The asterisks indicate very significant (** $p < 0.01$) difference between *PuWRKY75* transgenic lines and WT, based on Student's *t* test.

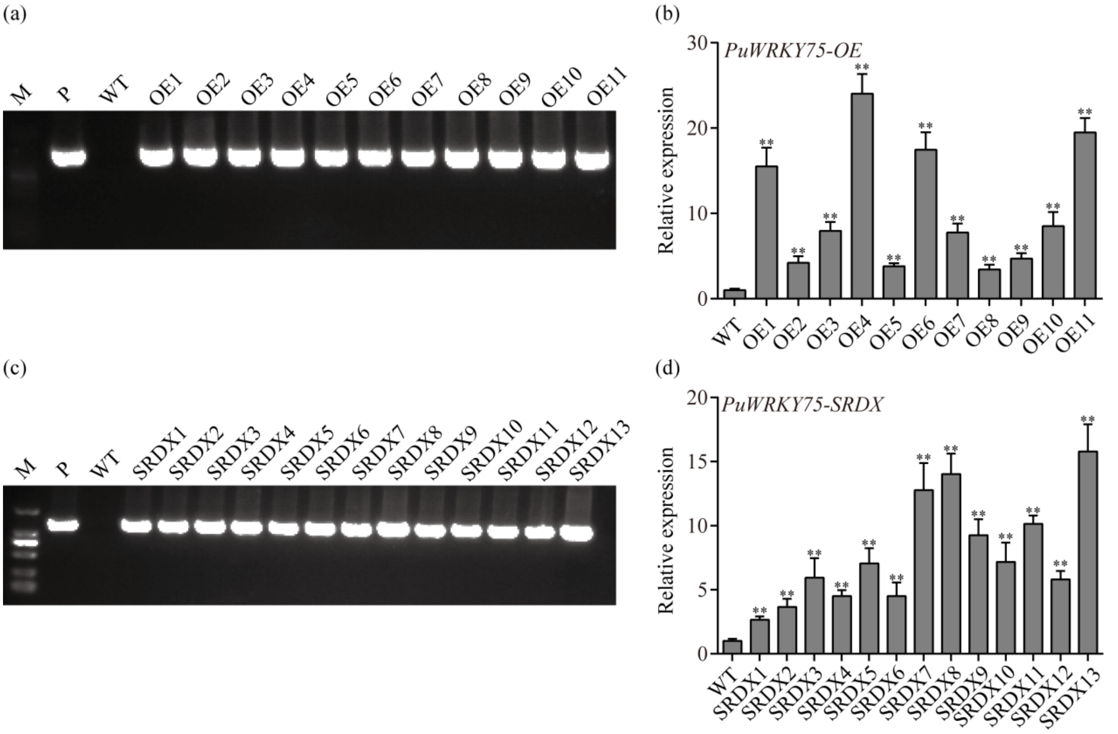


Figure S9 The promoter sequence of *PuLRP1* and *PuERF003*.

(a)

>PuLRP1pro
AAGTTACTACGGAGACGCGACATCGACTTCGCGTGTCGCCCTTAGTGCCTAACACCTTGGCTTATCTTGCTATATATATAAAAAAATATTGTTAA
CCAAAAAACACGTTGTTCTGATTCCTGTTGGATGATATGATGTTTGTTGACGCACATTGGTGAAAAATTAACTGGTAATTATCAAATATTAATTA
AATTTTTGTATTCGGTATATATAAGTTTCAGTATTTAATTACGAAGAAACTTTTTTTTTTTACTTCCTGTTTTGAAAATATAAAATTATACAAAAAA
ATATATTTATTTTATATATTTATTTTTTAAAATATTTTTGTCTCTACTATCTATATTTTTTATTATTTAATAATAATCCAATGCTACCTTACCTAGAAAG
AATTTCCTTATGAACAATTCTATATTGATACAGTGTATTAAAAAAATTATAAATTCTTCTAGATACTTAAATATTAAAAAAAAAACCACATATATCTC
CTGATCATGATGCAGATGAAGATTCAAATACCTATGAACAATTCTACGTAGATGAGGATTTATACATTAAGATTCGTGTCCTAGCGGTCCTGGCA
GGGAATTCTCTATCTCTGCACTCTAGATTCGAACCTTTGTGTGCATGTTTGTCATCCCCGTGGTGTCTTACCTGCCTATTGGGCTTGTAAAGTGT
TCAGTAGATCTGGAGATTATTCGTGGTGTGTATAAGCTGACCCGGACACCCGGGTTAAAAAAAAATTAAAATACCAAATATTTTGATTTTTATAA
TTATTACTTACAATCTTATAATTATTACTTGGAATCCTGTAACGACAGAGGAGGTCACCATTTCTTTCCACTGCCTCACCTGTGCCTGCCATTCC
ACTGCATCAAAAGGCTACTGGTAAGTAAATAAATTGAAGATGCATCATTACAGATAGGATAAAAGTGACTTAACTTTTATTTTTTTGTACTTTAA
AATTAACAGGCAATTATTGCTTCTTAAATTTGGTGGATTAATTATGAGAGGAGAAAAATAATTAAGCCATTACATTTTAGTTCGAGAAATATAAA
ATGAACATATATTTAACATTAAAATCCTATGAACTTGGATGTTTACTTTCAGAATAAATAAATAAATAAAAATCTATATATTTCCTTATTCATTGATT
AAATATATTCTTGAATCATTTTATGTATTAATCAATATGTTTTTGAGTGATTTTAAAAAACTTATTCATCTTATTATTAGTATAATGTATTTTAAAAAT
AATAATAAAATTGTATGAATTTTTGAATAAAAAGTTGAAAGATTTTCTAATGTATTTTTATACAAGGAGATATAATTTTTAATAATAGAATGCGTT
TTAAATTGAGTTTCAAAAAATTTATTTATTTTGAAAAAATATTAAATTAATATTTTTTAGTATTTTTTAATAATTTTAATATATTTTTAAATAAAAATA
TTTTCATGAATGGATATGTGGCTACAAGTAATAATTCCTAAAAAAAAAAGAAGAAGAAGAAGAGTGATTTACAGTTAGAAACAGAGGAATTT
CAGAACACAGTAGTTGACTACCAGTAGAAAAGGCAACTCTGCAACAAATCTCTCTCTCTGAGCAGCAATAATTGCCTTCATGTTGTACACTTC
TATATCATTGTAAGCTTAGCTAGCTAGCTAGCTCATATCCATCACTTAACTGCCATTGCAAGTCTTTTCTTTGTACTATACACAATCATCCACTTA
CATTCTTGATACAAACCATCAACCGTGGCCACTTCAACTCCTAGTACCCTCACCACCTCCAAAACATGGCCACCACCGCCACATCCAGTCACC
ACCACTACCTTCCCACCTGATCATACAAAC

(b)

>PuERF003pro
GTCAAAACTCCATACGCTCTAGGATTAAAATCATTTACTATAAATTATATTGTCCTGGTTACTGCAGCGAGGTCATACCCCTAGAACTCAGGCA
AATGATTGCTTCTAATATGGTAAAGTCCCTCTGCCATGGGAACAGCTCTGTCATACCCCTAGAACTCAGGCAAATGATTGCTTGTAATATGGTA
AGTCCCTCTGCCATGGGAGCAGCTCCCGACACAGAGAAGCAAACAGTCAAAATTAGCAACTGTAGAGATCGGGAGTGCTTCTACGATCAAT
TTTGTTTTTATTTTAAAATTTTTAATTTATTTTAATTATTTTTTTTATATTTTTTAATTATTTTAATTAGTTAATACTAGAAATAAATTTTAAAAATAA
AAAAATTTATTTTAATATATTTGTAGATAAAATTCACTTTAAAAAGTAATTTGACACCCACGTAACACACTACCCAAAGATAACATGAAGAATG
ATCTTAAAACTACAGTATACTTCAACTTGATCAGAAAATAATTCAATTATTATTTTATGGAAATTGATTCCTGAATACCAAAAAAAAATAAAATGC
TAGGGTCTCATTCTGTAGCATGGGGGTCCATGCTTGGCACATGAGTCTACAGTAAACATCAAGCATGGCCAAGAGGCAACCCAAACCAAAAC
CATGTACACTGTTTGTCTTCTATATATAATCTGTGGCGTATGCTCATGTTTCAAATTTGTGCGCCATATAAAACCAAAACGCAAGAAACAAAAG
AAAGAAGCAACCCTTGCTACAAGTCACGAGCCCATCATAGATTTATTAAGAATTGATAGCGTTTAGTTGCTCTCTCGTGAAGAAAAAAGAATT
CATTCAAGATCTATGTTATGAATAAATATGTTTTATATAATCTGTTTCTGTTTCGAAATACTAACCAAGCGTGAAATGAGTAAGCTAGTGAAAAT
GCTTTATGTTGAACAATCACCATACGCTTATTAAAAATTTAGAATCCTTCTGTCGAGAAACAGAGACATCTGGGTTGTATGCAAGAAAAATAA
TCTTTAGCTGCTCTTAGTGCATGTTGATTCTGTTATATAGTGAATAAGATTTGATCAAATGATTTGGATTGTTAGGGCAATTATTATGGTCTAAGA
ATCTTTAATAGTTGTGTCTCAACTTAGGATTGACCCAATTATGTCTGTTCCAAATTCTTGCATGAGCCTGTTTTTTTAACCTGTGGACCATTAAT
GACCAATGTAAAATTAAGAGAACTGTACATCTCACCACAACAGGGTAAATTACTCCCGTTTTTTGCATCTAAAATTGTGGCTTAAAACTCCCA
CATGCCCCCAATTAATTCTTTTCTTTTTTAAAAAATAAAAATAAAAAGCTAAAGCTACCTCAAATAAGCTTCGGGTCTCTTGGACATGAAGAA
AAGAGAGAGTTGTGGTGGAGTCGTTATCTTTGCTAGGCTGCTAACACATAACACTCACATGCATGAAGAGATGAATATCTACAAGACTATGAC
CTTAGATTTTCTTGAAGGTAGAGAGAAGCTCTCCAAAGCGAACAGTTGAATATTCTGATTTTTTGTTCTTTTCTTTTTTCAATCATGAAACAAA
AGCTATTACATATCTTGGTCATGTGAAATGAATTTGGATTTCAGTTTGAAATTCCACTTCACCAAAATATATTCAATTATTATCATACTTATAATAA
TAAAGCCAGCCAAGATAAAGAAAGGAATAGAAAGACCTAAACCCCAAGTTGGGGACCCACTTCTTAAATTGGTTGCCAGACGAGCTTGCCT
TATATCTTTCAAACAAACCCAGCTACAAAAACCCCTCTTCCCCACCCCTTCATCAGCATCACTCCCTTGCTTTCCAACATTCTCCCTTTTCTTA
CAGGCATTTTCACAAACACTTTCTCAAAGAAACGTTTCTTTTTTTTCACGAAC

Figure S10 PCR and RT-qPCR verified the expression level of *PuLRP1* and *PuERF003* in *PuLRP1* and *PuERF003* transgenic lines, respectively.

(a), (c), (e) and (g) PCR confirmation of *PuLRP1* and *PuERF003* transgenic lines, respectively. M represents DNA marker, P represents PCR product with pBI121-*PuLRP1,* pBI121-*PuERF003*, *PuLRP1-SRDX* and *PuERF003-SRDX* plasmid DNA as template, #1-14: PCR products with genomic DNA from resistant seedlings.

(b), (d), (f) and (h) Quantitative analysis of the expression levels of *PuLRP1* and *PuERF003* transgenic lines, respectively. *PuActin7* and *PuUBQ10* served as internal control. The asterisks indicate very significant (** $p < 0.01$) difference between *PuLRP1* and *PuERF003* transgenic lines and WT, based on Student's *t* test.