

Supporting information

Supplemental Table 1. Identification of flavonoids in white lupin by UPLC/MS and MS/MS analyses.

peak	compound	t _R (min)	MS/MS	Leaves (mg/g)	Roots (mg/g)
1	2'-hydroxygenistein 7-O-glucoside	8.259	447.0;285.0	0.22 ±0.04	0.24 ±0.02
2	2'-hydroxygenistein 4'-O-glucoside	8.441	447.0;285.0	2.03 ±0.41	5.19 ±0.80
3	genistein 7-O-glucoside	10.254	431.0;268.0	1.62 ±0.17	3.48 ±0.13
4	kaempferol 3-O-glucoside	10.579	447.0;284.0	2.04 ±0.72	-
5	kaempferol 3-O-galactoside	10.935	447.0;284.0	^a +	-
	isorhamnetin 3-O-glucoside		477.1;314.0	^a +	-
6	isorhamnetin 3-O-galactoside	11.116	477.1;314.0	4.38 ±0.55	-
7	2'-hydroxygenistein	13.943	285.0;217.0;175.0	0.24 ±0.11	0.62 ±0.07
8	genistein	16.66	269.0;224.0;133.0	0.06 ±0.03	0.51 ±0.13
9	luteone	22.957	353.1;285.1;219.0;133.0	0.03 ±0.01	0.11 ±0.02
10	8-prenylkaempferol	23.199	353.1;337.0;298.0;253.0;164.0	-	^a +
	putative compound		351.0;283.0;199.0;151.0	-	^a +
11	wighteone	25.424	337.1;281.0;201.0	^b +	0.11 ±0.01

Note: ^a: double compounds and could not be separated for quantification; ^b: the amount is too low and could not be quantified; -: not detected. The information on isoflavonoid compounds were highlighted in bold.

Supplemental Table 2. Primer name and sequences used in the present study.

Gene name	mRNA accession number	Primer name	Primer sequence (from 5' to 3')
Protein Expression in yeast			
LaPT2	LAGI02_4454	LaPT2Spe1F	GGACTAGTATGGGTTTTGTGCTTGCAGCTAC
LaPT2	LAGI02_4454	LaPT2Xho1R	GTGCTCGAGTCATCTAAATAAAGGTATGAGG
Subcellular Localization			
LaPT2	LAGI02_4454	LaPT2SubLSal1F	ACGCGTCGACATGGGTTTTGTGCTTGCAGCTAC
LaPT2	LAGI02_4454	LaPT2SubLBamH1R	CGCGGATCCTCTAAATAAAGGTATGAGG
LaPT2	LAGI02_4454	LaPT2SLBamH1R ₈₇	CGCGGATCCTTCATTTGATTTTCCAGAGGCT
LaPT2	LAGI02_4454	LaPT2SLSal1F _{Δ1-87}	ACGCGTCGACATGTATGAAACCCAAGATCTTGATC
qRT-PCR Analysis			
LaPT2	LAGI02_4454	LaPT2 qF	GCAGCTACTTCTTTTCCCAAAG
LaPT2	LAGI02_4454	LaPT2 qR	ATTTCTCTTGTCTCCCCCTTTG
Actin		LaactinRTF2	TGGACGACCTCGTCATACTG
Actin		LaactinRTR2	AAGCATCCTTCTGACCCATC
LaIFS2	rLAGI02_1754	LaIFS2 qF	CATCCACCACTTCCTGTGGTT
LaIFS2	rLAGI02_1754	LaIFS2 qR	ACACTAAGTCCTGGCCTCTCT
LaIFS1	LAGI02_33262	LaIFS1 qF	AGCCGTGGCAACAGACTATG
LaIFS1	LAGI02_33262	LaIFS1 qR	TCCCCTCAGATCAACTGGGT
LaMyb4	LA02_38569	LaMYB4 qF	ACAAGGGAAGTGGTGGTTCAA
LaMyb4	LA02_38569	LaMYB4 qR	CCAAGGCCTCCTCAACACTAC
LaMyb3	LA02_38159	LaMYB3 qF	CGTTGTCGGAGACACGGAAT
LaMyb3	LA02_38159	LaMYB3 qR	TAACTCAACGCCCCACCAAC
LaMyb1	LA02_44741	LaMYB1 qF	GTGAGGACAGTGGTGCAGTT

LaMyb1	LA02_44741	LaMYB1 qR	CTTTCCAAAGCCAAGTGAGCA
LaMyb2	LA02_2514	LaMYB2 qF	TGCCTCAAAGGAATGCAACTG
LaMyb2	LA02_2514	LaMYB2 qR	GCTCCTCCTTAAGGGTCACAT
LaF3H1	LAGI02_43731	LaF3H1 qF	AAGCAGTGCAGGATTGGAGAG
LaF3H1	LAGI02_43731	LaF3H1 qR	TGGTTCCTCCAACATAGGCTTC
LaF3H2	La02_41747_71057	LaF3H2 qF	AGAAGATTGTGGAGGCGTGT
LaF3H2	La02_41747_71057	LaF3H2 qR	TCCACCCTTCTGGCTTGTCT
LaF3H3	LAGI02_54973	LaF3H3 qF	AGCCGTCTATCCATAGCCAC
LaF3H3	LAGI02_54973	LaF3H3 qR	TTGGCCATGTCTGAAGTTTTGC
LaFLS1	LAGI02_45697	LaFLS1 qF	GGCATCACAACGGTTCATGG
LaFLS1	LAGI02_45697	LaFLS1 qR	ACACTTTGTCTACCACGCCA
LaFLS2	rLAGI02_2306	LaFLS2 qF	AAGGGTACAAACTGTGGCATC
LaFLS2	rLAGI02_2306	LaFLS2 qR	TGATGCCTGGTTGTTCTGTCT
LaFLS3	rLAGI02_904	LaFLS3 qF	AAGCTGCAGGTGGAGATAGC
LaFLS3	rLAGI02_904	LaFLS3 qR	TTGCACGTCATTGGGGACAA
LaF3'H1	rLAGI02_34328	LaF3'H1 qF	GTTGGCCGGAGCTCTCAATA
LaF3'H1	rLAGI02_34328	LaF3'H1 qR	ATTGGGGTTCACGTGCTATGG
LaF3'H2	LAGI02_35407	LaF3'H2 qF	AATTTCTCTAGCCGGCCACC
LaF3'H2	LAGI02_35407	LaF3'H2 qR	CAGTGAGTCTGCCTCCATCC
Over-expression in			
<i>Arabidopsis thaliana</i>			
LaPT2	LAGI02_4454	LaPT2KpnIF	CGGGGTACCATGGGTTTTGTGCTTGCAGCTAC
LaPT2	LAGI02_4454	LaPT2SalIR	ACGCGTCGACTCATCTAAATAAAGGTATGAGG

LaPT2	MGFVLAATSFPKAPSTSGRSSWNSKEYTKN..YYASSHVTTLWHKGTGIIQREPCFMMAWPQNLKLHCKVK	69
SfN8DT	MGSMLLASFPGASSITTTGGSCLSRSKQYAKN..YDASSVVTTSWYKKRKIQREHCAAFISKHNLKQHYKVN	68
GuA6DT	MAKNSLNPISFFGQKERHSPSFGGNIWQSNCTKN..YYASSYAPKASWHKKNIQREYFFLRFKQSSSNHLYKDI	73
SfFPT	MGSMLLASFPGASSITTTGGSCMRSKQYAKN..YNASSVVTTLWHKKGKIQREHCAVIFSKHNLKQHYKVN	68
SfILDt	MGFVLPASFPSSSITTTG.....SYCTTLWHKKSEKIQREYCVMLSSSHNLKRRHKVI	52
LaPT1	MSAMLASCTIPSSIKAGNRPSSKQCGRT..YYASSNVPTLWHKTEKIQREHCAAMSS..NSLQHRCKVI	67
SfG6DT	MGFVLPASFPGASSITTTGGSCMRSKQYAKN..HYASSVLTLLCHKTGENKREYRFMMSSQPNLRHHYRIM	68
LjG6DT	MMQSLVGLVSLNASSFTTGGNLWQSTKHGANKNKYYESGNLLRSKHRMKNKNCYTSSYAPKASQNKGNPDEYKLLRFHEPSTNCPKCI	90
GmG4DT	MDWGLAISSHKKTYSVTTGGNLWRSKHTTKNIYFASSWISKASHKRETOIEHNVLRAEQPSLDHHYKCI	70
GmG2DT	MDSGVSISSTASCTITGGNLWRRKHSTNMIIYASSCASKASKYKKKTQIECNILRSQQSSLNHHYKRI	69
LaPT2	GGDKRN.....YVMNAAFCKSNFYETQDLDORNWGTLLINALHVFKKFIRFATLTLGATLTLTAVERISDSSAAAYFTICLLQV	150
SfN8DT	EGGSTNTSKCEKKYVNNALIEQSFYEBPQTRDPESIWDSVNDALDIFYKFCRPMAMFTIVLGATFKSLVAVERISDSSAFFITGLQV	158
GuA6DT	EGGSTYR...ECNRKYVVRAPGPSFSESPAFDSKNILESVKNFINVFKLISPMAMIAAALSITSASLLAVERISDSSPQFPITGLQV	160
SfFPT	EGGSTS...KKREKKYTVNAIEESFYEYEPQVRDPESIWGSVNDALDIFYKFCRPMAMFTIVLGATFKSFVAVERISDSSITFTITGLQV	155
SfILDt	HRGSSCQ...ECERKYVNNATSGQLFYEYEQATDIKSNWDSIKDALNVFYSFMRPMASATAAMGATSVSLLAVERISDSSLPFFITGLQA	139
LaPT1	EDGPKYQ...QWKRKCTINALEQSFSEFESQAQYKKSMKDSVKDGLVAFYETREPMASATPITILEATCMBLLAVERISDSSLIFFKQWVOT	154
SfG6DT	EGGSTCQ...ENEKKYIVKATSKQTIEYEPHAQHSKSIWDSIKNAFDATYFRRPMAAIEAALGATSIISFLAVERISDSSVVFITGLQV	155
LjG6DT	KRGSTYQ...EYNQKYVLRASTQPFEPHPPFDSKNITLDSGKNIFVALYKFSIPMAIFVRMLSTISASLLAVERISDSSLPFTITGLQV	177
GmG4DT	RGGSTYQ...ECNRKFVVKAIKQPLGFEEAHASNPKNILDVKNVLSAFYWFYSYPTMTIGTILCFSSSLAVERISDSSLSFLITGLQV	157
GmG2DT	EGGATYQ...DCKKYVVKALPEPTFDSBCASNPNVVDSSAKKILDVYHFCYPMSTIAITLCASSLLAVERISDSSSFLITGLQA	156
LaPT2	MVVSQCMQVFMAGNOLYDVEDCKNKPYLPLVSGELPFKNGVITVATTFILGHLFPLIISGPTFWFSFVSSSLAIAACADLPILLRWKR	240
SfN8DT	VVAIVICHIHFGVGNOLCDIEIDCKNKRPDLPLASGKLSERNVVIITASSLLILGLGFAMIVSSTPFWTIVFISCMVASANNVLDPLLRWKR	248
GuA6DT	LIPNLFMCVYMAGINOLCDIEIDCKNKPHLPLASGELISFTTGVIIITASSFIVSLWLGSIIVSSTPFWTIVFISCMVASANNVLDPLLRWKR	250
SfFPT	VVAIVICHIHFGVGNOLCDIEIDCKNKRPDLPLASGKLSERNVVIITASSLLILGLGFAMIVSSTPFWTIVFISCMVASANNVLDPLLRWKR	245
SfILDt	VVFSFIVNIFNCGNELCDVIEDCKNKPNLPLVSGELSFRTGVIIITASSLLIMSFGLTLIVSSTPFWFSQFASLLAAAYSINLPLLRWKR	229
LaPT1	VVATLLMIIVNCGNELCDIEIDCKNKPHLPLTSCALSIKAAIAIVASAFGLWFSWSGSPFWFNVLVNNVLAIVFVSVLDPLLRWKR	244
SfG6DT	VVASFFMNIFHCGINOLCDIEIDCKNKPYLPLASGELISERNVLIIVASSLLMFCGLAWIERSTPFWGFFVCAMLTAAAYSINLPLLRWKR	245
LjG6DT	MVAHLFMGIYVGGNOLFDGIDCKNKPYLPLASGKLSFTTGVIIITVCCLLSFSGLWMFCSAPLIISLLSCAVVTSVSNANVPLLRWKR	267
GmG4DT	VLPQLFIEIYLCGNOLYDIEDCKNKPHLPLASGQFSFTKGVITISAFALISFGFTWITGSNPLICNLVVIASSWTAAYSIDVPLLRWKR	247
GmG2DT	LVPHLFVAVFANVNOVFYEDCKNKPYLPLASGELSETTAVFIAASLLIMSEFWLSLVIISNPLIINNVLTSVWNVYSINVPLLRWKR	246
LaPT2	HSATLALNYIDLGGVKELGYVLHNOTYVEKKRPPTFSRPLIFCMAMSVFAIITATFKDIDDMEGDKKEGKLSLHLGKRVPVFIQVSL	330
SfN8DT	YFVLTAINFIADVAVTRSLGFFLHNOTQVEKKRTTTFRPLIFCTAIVSIYAIIVIALEKDIIDMEGDKKEGKLSLRLGKRVPVFIQVSL	338
GuA6DT	HPALAAACIIATWGFIFIGYFLHNOTQVEKKSAVSRPVVESTIFTMSSFFSLVIALEKDIIDIEGDKAGVQSFSASLGKRVPVFIQVSL	340
SfFPT	YFVLTAINFIADVAVTRSLGFFLHNOTQVEKKRTTTFRPLIFCTAIVSIYAIIVIALEKDIIDMEGDKKEGKLSLRLGKRVPVFIQVSL	335
SfILDt	YFILAATSILTNAVAVPLGYFLHNOTQVEKKRATFPRLNFCIAILSLFFVVIALLKDIIDIEGDKKEGQSLAVRLGKRVPVFIQVSL	319
LaPT1	SSFLITAVYILTNIGVPIIGSFLHNOTQVEKKRAATLFRSMLLSTTVLIFICIVISMKDIIDMEGDKKEGKLSFALSGLGKRVPVFIQVSL	334
SfG6DT	SSMLAANIFVNAVGLRPLGYFLHNOTQVEKKRTTTFRPLIFCMAILSLFFVVIALLKDIIDTDEGDKKEGIRSLSAQLGKRVPVFIQVSL	335
LjG6DT	HEVSAALSIVATYAVIFIPDFLHNOTQVEKKRPVFRSLITFVTVFMSLYSMGIALIKDIIDVEGDKKEGITYSPAREGKRVPVFIQVSL	357
GmG4DT	YFVLAACMCMISTWALALPISYTHHNOTVVKRRIGFPRSLCFLVAFMTFYSLGLALS KDIDVEGDKKEGIDSPAVRLGKRVPVFIQVSL	337
GmG2DT	HELATICTISVWAIFILPITFPLHNOTQVEKKRIVFRSLIFVYVFMIFYSLGMAILKDIIDVAGDAAGIDTALRLGKRVPVFIQVSL	336
LaPT2	LQMAYVAILMCTLSPLVWVKIAMGLCGHILASLVSYANSVDLKSNPAIQSFYMEIWK..LLTVEYFLIPLER	402
SfN8DT	LEMAYGVITILVATSPILMSKIIITVLGHAVALVWVHAKSVDLTSNVVLHSFYMEIWK..LHTAEMFLIPLER	410
GuA6DT	LEMAYGVAILMCTSSCLMSKIIITVLGHAVALVLFWRAKSINLKSASIASFYMEIWK..LLYAEYFLVPLER	412
SfFPT	LEMAYGVITILVATSPILMSKIIITVLGHAVALVWVHAKSVDLTSNVVLQSFYMEIWK..LHTADYFLIPLER	407
SfILDt	LEMAYGVITILVATSPILMSKIIITVLGHAVALVWVHAKSVDLTKNNDKSYKSFYMEIWK..LICAEYFLIPLER	391
LaPT1	LQMSYGVGILVATSPILMSKIIITVVGHAVALVLCWRAKSVDPKSKDSQSFYMEIWK..LLYAEYFLIPLER	407
SfG6DT	LQMAYGITILVATSPILMSKISMVVLGHAVALVLCQVKSVDLKNNDALQSFYMEIWK..LLTVEYFLIPLER	407
LjG6DT	FELAFGIALMVATSPILMSKISMVVLGHAVALVWVHAKSVDLTKNNDKSYKSFYMEIWK..LLFAMMFLIPLER	429
GmG4DT	FEMAFVGILVATSPILMSKIIITVVGHAVALVWVHAKSVDLTKNNDKSYKSFYMEIWK..LLYAGFFLMALR	409
GmG2DT	FEMAFVGILVATSPILMSKIIITVVGHAVALVWVHAKSVDLTKNNDKSYKSFYMEIWK..LLYAEYFLIPLER	408

Figure S1. Sequence analyses of the deduced prenyltransferases from *Lupinus albus* with other closely related flavonoid-specific prenyltransferases in Leguminosae. Multiple sequence alignment of the two deduced prenyltransferases from *Lupinus albus* with other related flavonoid-specific prenyltransferases. Identical amino acids are shown in black background and similar amino acids are in gray background. The two conserved NQxxDxxID and KDI/LxDxE/DGD motifs are boxed, and the transmembrane domains were indicated in solid lines.

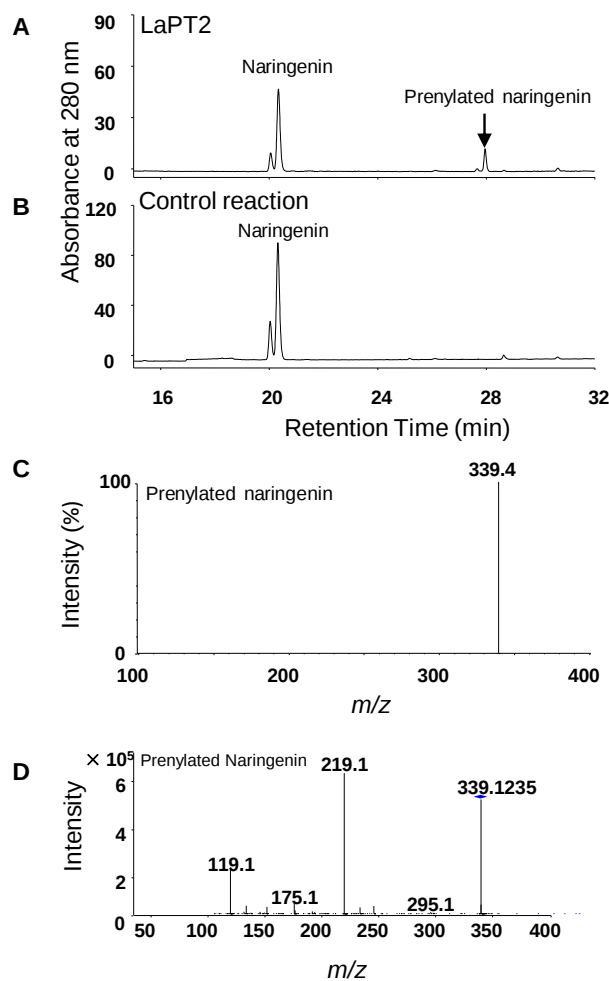


Figure S2. Identification of the enzymatic product of recombinant LaPT2 protein by HPLC, UPLC/MS and UPLC/MS/MS analyses with naringenin as substrate.

(A-D) Representative HPLC chromatographs of the enzymatic reaction containing naringenin, DMAPP and microsomal fraction of yeast expressing LaPT2 (A), control reaction (B), mass spectrum of the enzymatic product of recombinant LaPT2 protein determined by UPLC/MS (C), and MS/MS spectrum of the enzymatic product (D), respectively.

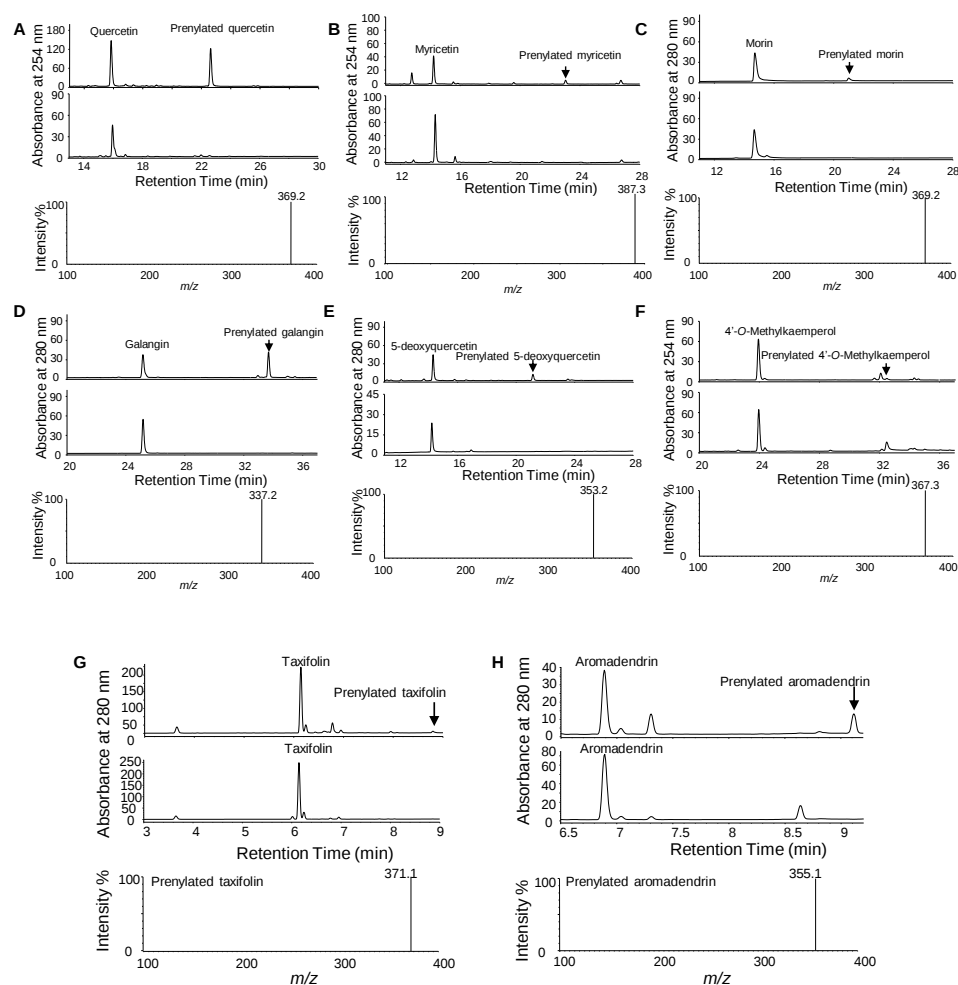


Figure S3. Identification of the enzymatic product of the recombinant LaPT2 protein by HPLC and UPLC/MS analyses.

(A-H) Representative HPLC chromatographs of the enzymatic reactions containing quercetin (A), myricetin (B), morin (C), galangin (D), 5-deoxyquercetin (E), 4'-O-methylkaempferol (F), taxifolin (G), and aromadendrin (H), DMAPP and microsomal fraction of yeast expressing LaPT2 (upper panel), control reaction (middle panel), tandem mass spectrum of the enzymatic product of recombinant LaPT2 protein determined by UPLC/MS (lower panel), respectively.

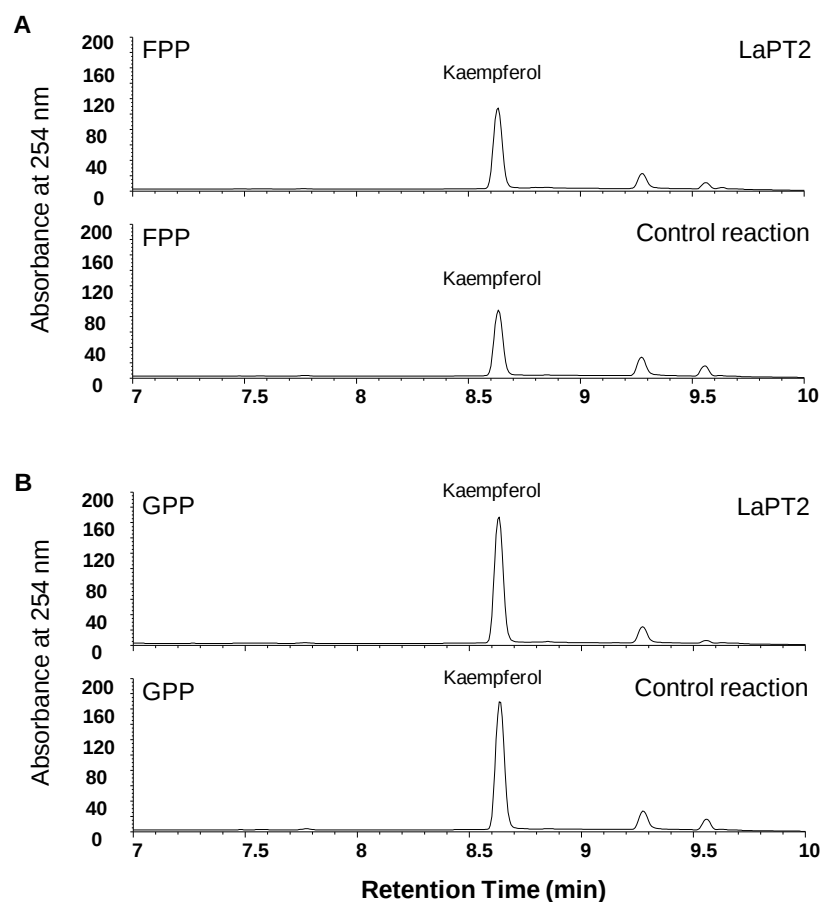


Figure S4. Analyses of the enzymatic product of the recombinant LaPT2 protein by HPLC with FPP and GPP as prenyl donors.

(A-B) Representative HPLC chromatographs of the enzymatic product containing kaempferol as substrate, FPP (A), GPP (B) as different prenyl donor, with microsomal fraction of yeast expressing LaPT2 (upper panel) or control reaction (lower panel), respectively.

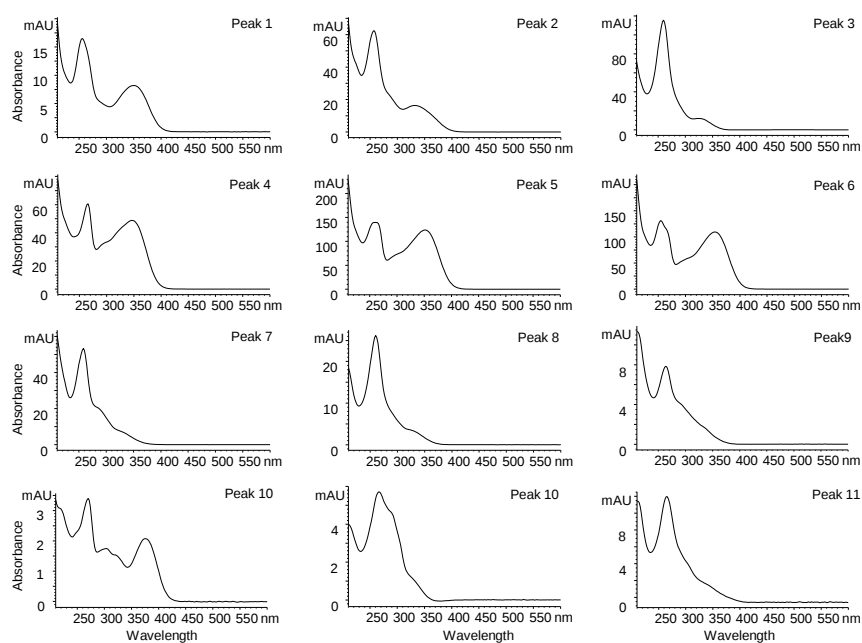


Figure S5. Identification of the major flavonoid compounds in white lupin by UV chromatograph analysis.

UV chromatographs of flavonoid compounds from white lupin for peaks 1-11 as shown in Table S1.

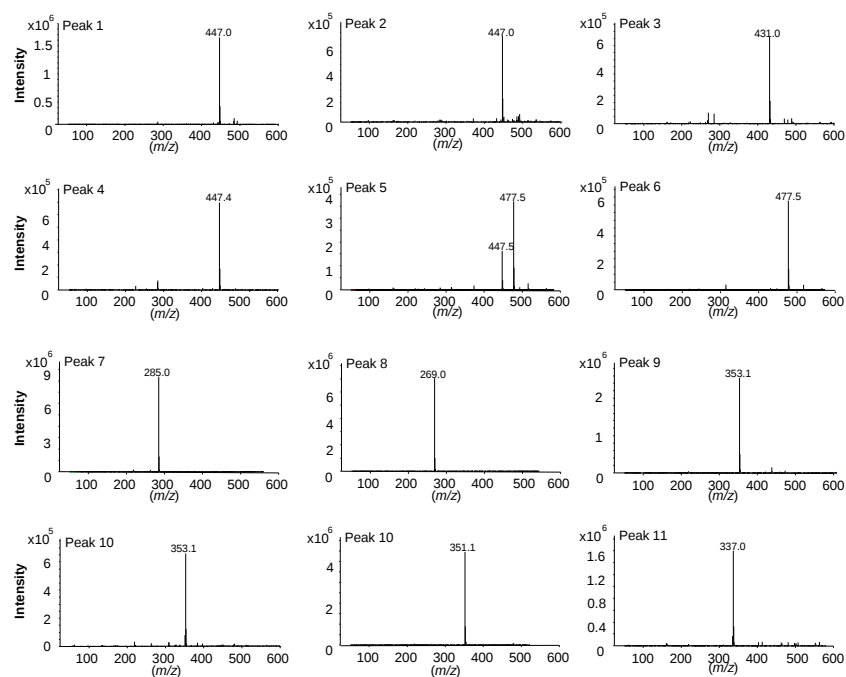


Figure S6. Identification of the major flavonoid compounds in white lupin by mass spectrum.

Mass spectrum of flavonoid compounds from white lupin for peaks 1-11 as shown in Table S1.

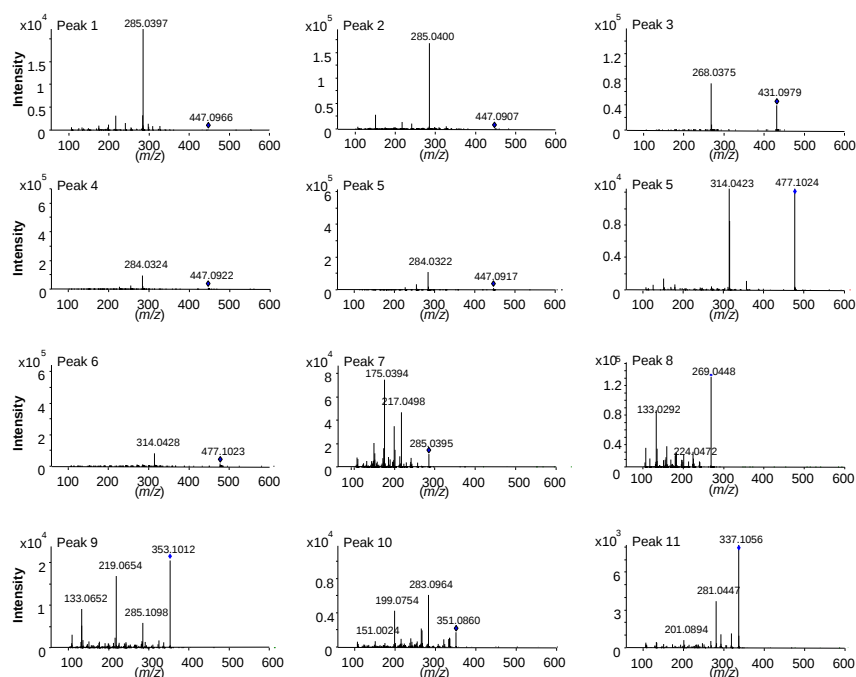


Figure S7. Identification of the major flavonoid compounds in white lupin by tandem mass spectrum.

MS/MS analyses of flavonoid compounds from white lupin for peaks 1-11 as shown in Table S1.

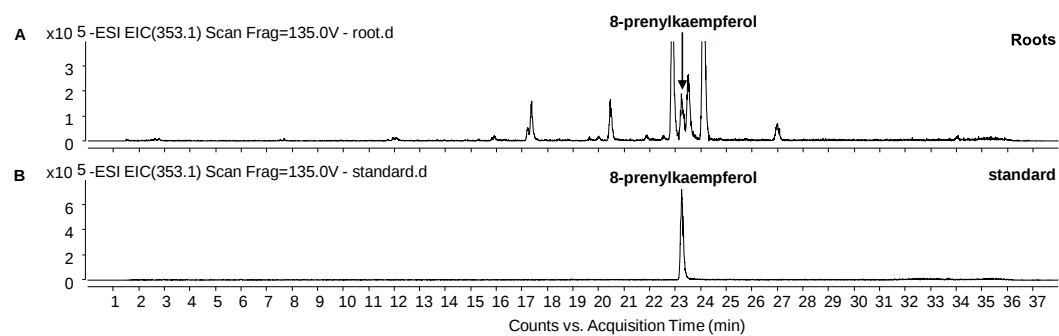


Figure S8. Identification of the 8-prenylkaempferol in roots of white lupin in comparison with the authentic standard by UPLC/MS analysis.

(A-B) Mass spectrum of the roots metabolites by searching the **ion** fragment with m/z of 353.1 under negative **ion** mode in the roots sample (A) in comparison with the authentic 8-prenylkaempferol standard (B).

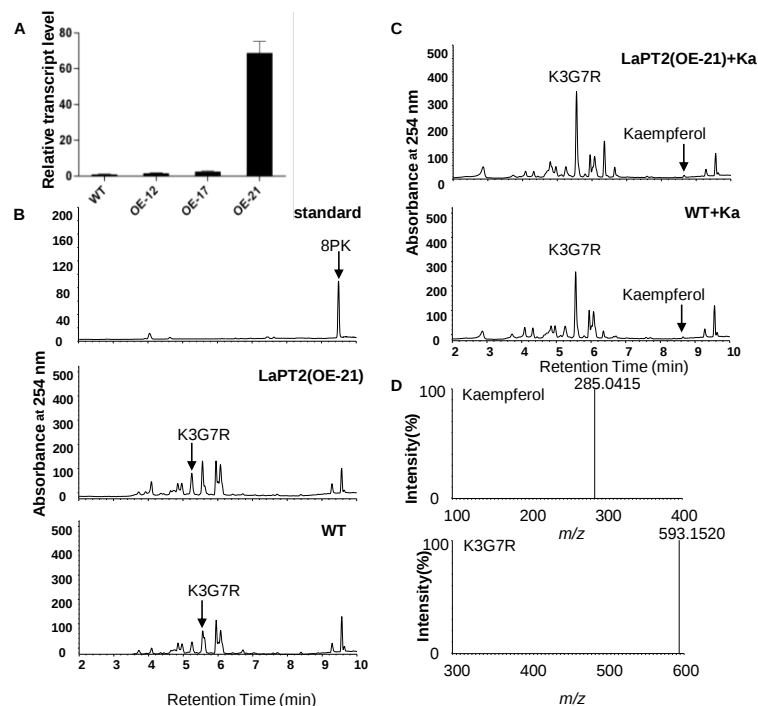


Figure S9. Analyses of the *A. thaliana* plants over-expressing *LaPT2* gene in comparison with the wild type control.

(A) Detection of expression level of *LaPT2* in transgenic *Arabidopsis* by qRT-PCR with triplicates. (B) Representative HPLC chromatographs of the flavonoid profiles in seedlings of the transgenic lines (LaPT2, middle), and the wild type (WT, lower) in comparison with standard 8-prenylkaempferol (upper). (C) Representative HPLC chromatographs of the flavonoid profiles in seedlings of the transgenic lines (LaPT2, upper), and the wild type (WT, lower) feeding with kaempferol. (D) Mass spectrum of kaempferol (upper) and K3G7R (lower).