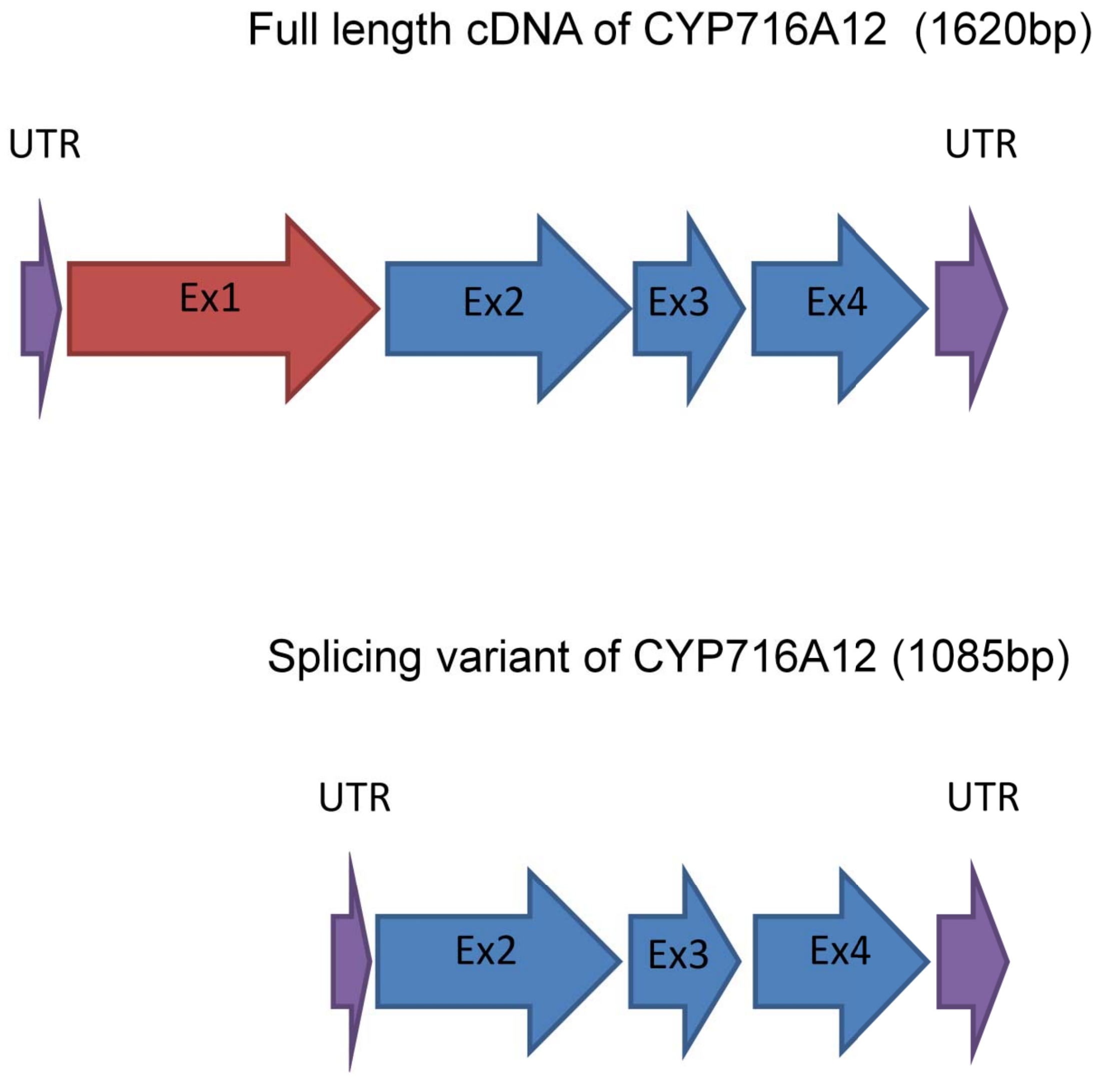
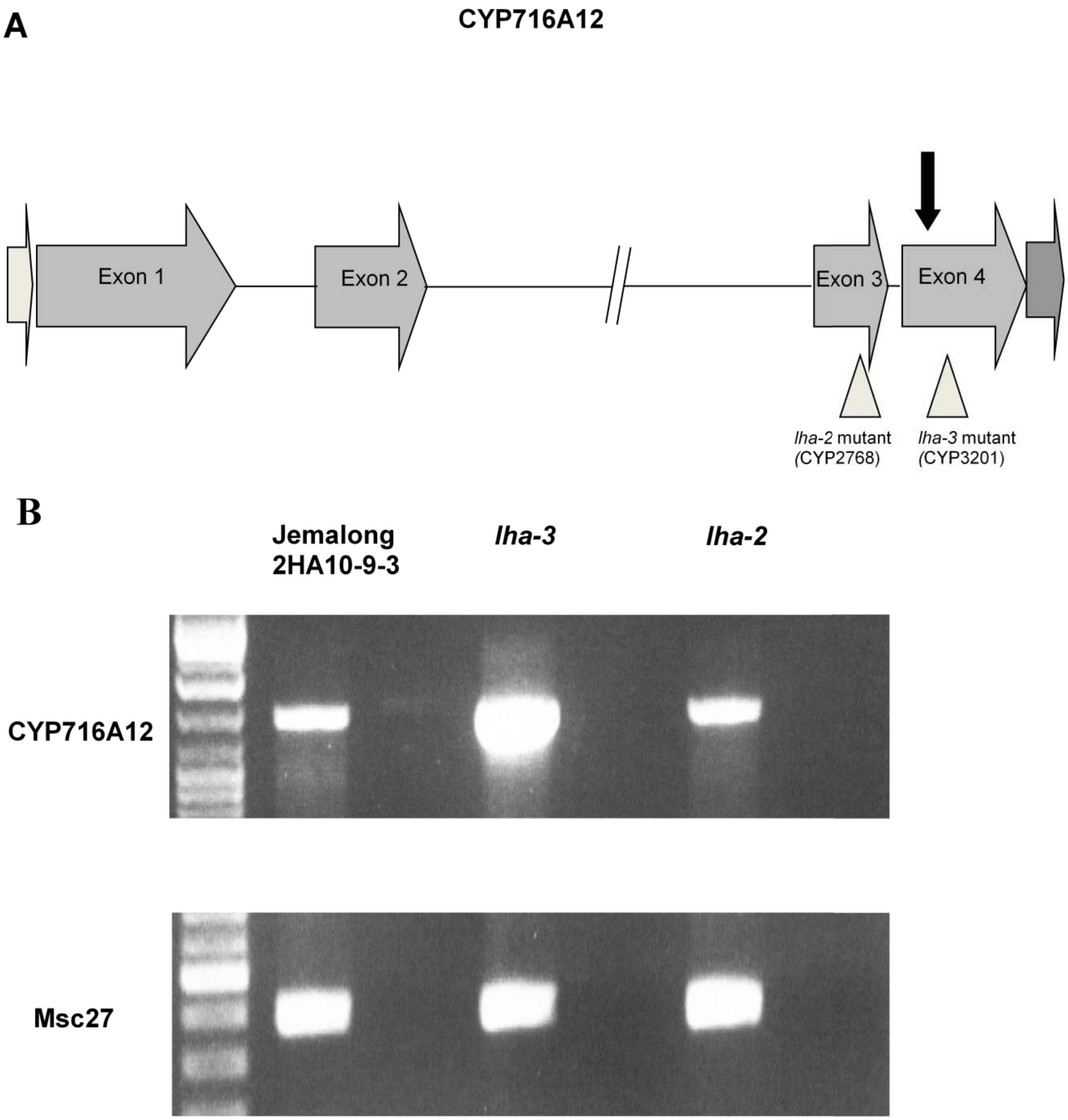
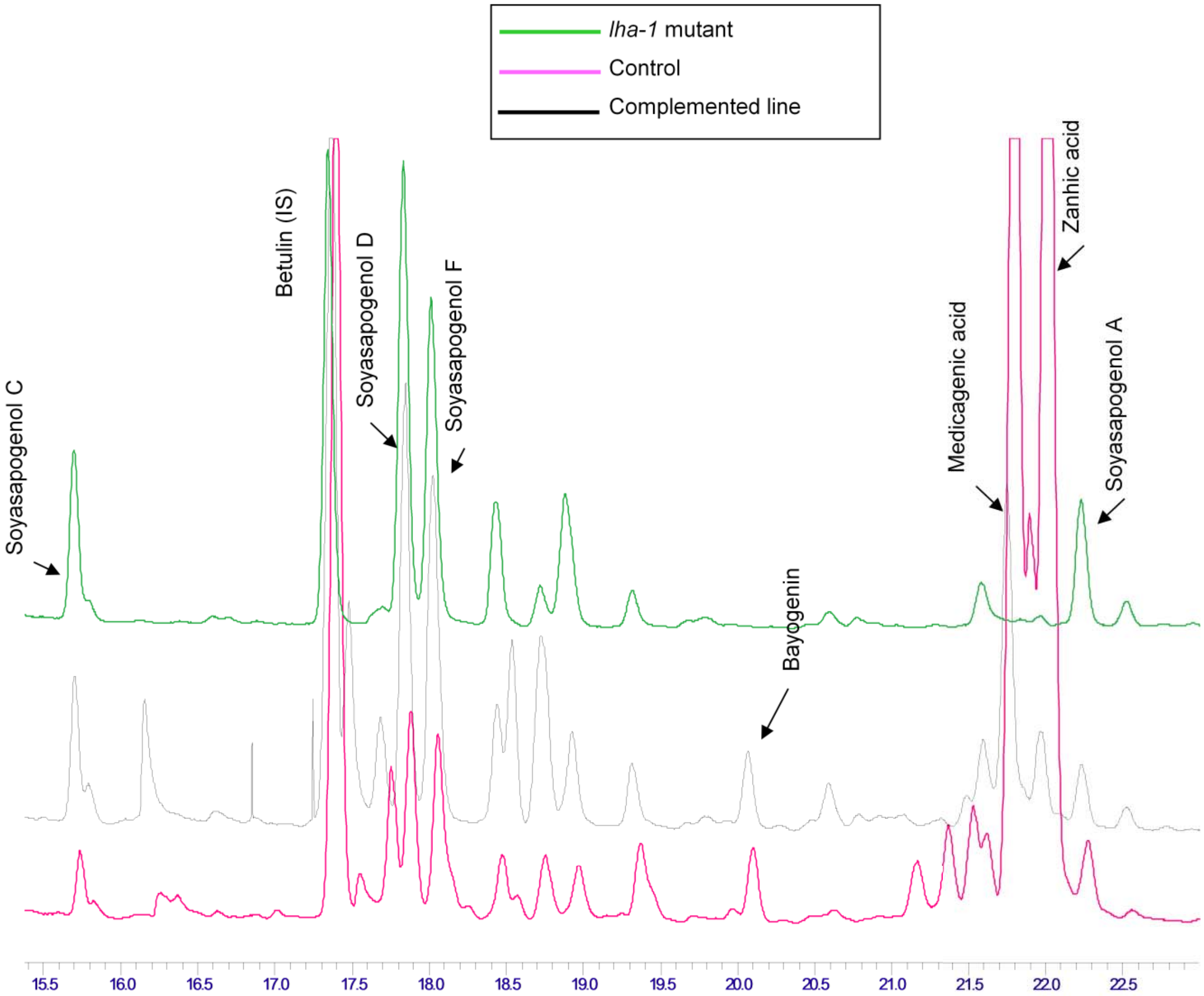


Supplemental Figure 1. CYP716A12 splicing variant

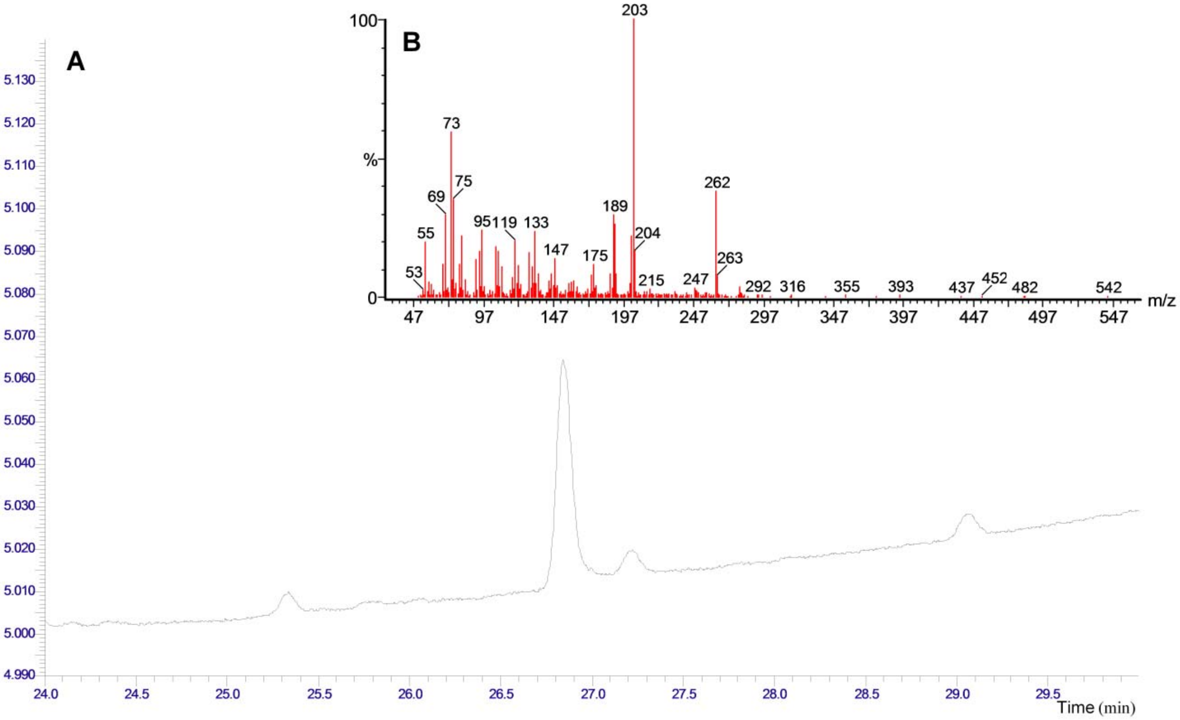


Supplemental Figure 2. Mutant alleles for CYP716A12 in TILLING collection.
(A) Alleles of CYP716A12 obtained from the TILLING collection (solid triangle): *lha-2* CYP2768 (P355→L) and *lha-3* CYP3201 (W449→ stop) lines. The position of the heme cysteine ligand is indicated (black arrow).
(B) RT-PCR analysis of transcript level of CYP716A12 gene in *lha-2* and *lha-3* mutants.

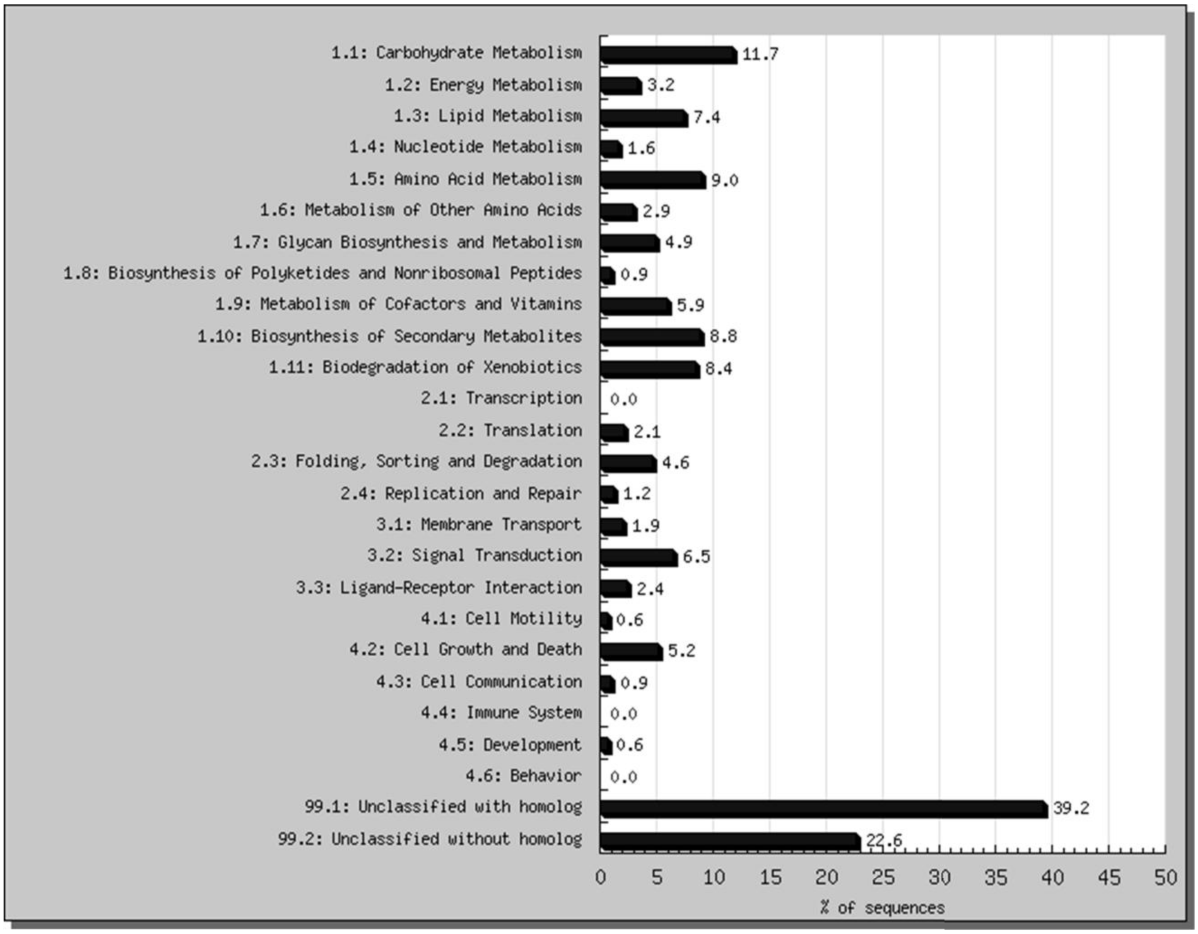


Supplemental Figure 3. GC/FID chromatograms of sapogenins of control line (E113) (red), *lha-1* mutant (green) and one transformed line derived from complementation of *lha-1* mutant (black). Identification of soyasapogenol B was achieved considering all the artefact compounds detected (soyasapogenols C, D and F).

Supplemental Figure 4. Immunoblot analysis of the CYP716A12-harbouring strains. Lane 1, FLAG-BAP™ Fusion Protein (SIGMA-Aldrich); lane 2, WAT11 expressing pESC-HIS under galactose induction; lane 3, WAT11 expressing pESC-HIS-CYP716A12:FLAG under galactose induction.



Supplemental figure 5. GC-MS analysis (A) and mass spectrum (B) of the standard oleanolic acid.



Supplemental Figure 6. Bar charts showing GO annotations of the *M. truncatula* probesets modulated in the *lha-1* mutant.

Supplemental Table 1. T_2 lines segregating for the Lha phenotype: segregation ratio and χ^2 value.

	Wild type	LHA phenotype	χ^2
E25-1	25	5	1.11
E25-2	25	5	1.11
E25-3	-	10	
E25-4	26	4	2.18
E25-5	23	7	0.04
E25-6	-	14	
E25-7	21	9	0.40
E25-8	29	-	
E25-9	29	-	
E25-10	-	30	

χ^2 value is within 95% confidence value for a 3:1 segregation ratio

Supplemental Table 2. Comparison of the *lha-1* mutant line (E25-10) and the full sib control line (E25-08). The interaction line x block was used as error term.

Characters	Control	Mutant
Germination (dd)	5.00	10.60*
Flowering (dd)	58.79	56.85*
Sum of length (cm) main stems	393.08	201.77*
branches	687.51	209.60**
Root DM (mg)	185.77	76.96*
Nodul Fresh Weight (mg) absolute	10.18	19.73†
per unit of root DM	56.62	274.47‡
Nodule number absolute	4.33	6.47*
per unit of root DM	0.025	0.117 ns
Pod number per unit of stem length	0.070	0.061 ns
Pod fertility (number of seeds/pod)	5.27	4.73 ns

significantly different at: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{\dagger}P = 0.0687$, $^{\ddagger}P=0.0747$.
ns: not significant , DM: dry matter.

Supplemental Table 3. ANOVA of sapogenin content (GC/FID) in control plants sampled in different organs at different phenological stages: test F and significance.
Only significant contrasts are reported.

Source of variation	SoyaA	SoyaB	Spg non-hem	Olea	Hed	Bayo	Med	Zan	Spg hem
Stage	1.5 ns	1.2 ns	1.4 ns	0.12 ns	2.7 ns	2.4 ns	16.1****	4.7*	11.9****
Organ	11.8****	26.1****	22.9****	34.7****	79.4****	54.2****	0.7 ns	232.3****	32.5****
StagexOrgan	0.4 ns	0.7 ns	0.7 ns	0.12 ns	1.4 ns	2.6 ns	3.4*	0.2 ns	2.0 ns
Contrast									
S1*vs*S2/ root					9.0**		32.6****	5.3*	21.2****
S1*vs*S3/ root					6.8*	13.6***	25.0****		17.1****
S2*vs*S3/ root						8.4**			
L*vs*R/ S1		13.9***	13.4***	19.4****	21.8****	10.6***		123.0****	21.4****
L*vs*S/ S1								103.1****	17.7****
R*vs*S/ S1		12.0***	10.8***	19.4****	21.3****	17.5****			
L*vs*R/ S2	5.2*	20.6****	18.7****	13.5***	53.9****	16.3****	4.9*	111.7****	
L*vs*S/ S2								119.6****	22.4****
R*vs*S/ S2	10.3***	21.2****	18.4****	13.5***	51.5****	21.8****	6.4*		9.2**
L*vs*R/ S3	5.8*	5.6*	4.5*	19.4****	46.8****	48.2****		122.3****	5.6*
L*vs*S/ S3								117.6****	23.6****
R*vs*S/ S3	9.6**	8.7**	6.9*	19.4****	51.5****	61.8****			6.3*

* P≤0.05; ** P≤0.01; *** P≤0.005; **** P≤0.001; ns: not significant

SoyaA: soyasapogenol A; SoyaB: soyasapogenol B; Olea: oleanolic acid; Hed: hederagenin; Bayo: bayogenin; Med: medicagenic acid; Zan: zanhic acid; Spg hem: haemolytic sapogenin; Spg non-hem: non-haemolytic sapogenin.
S1, S2, S3: stages 1, 2 and 3 respectively. L: leaves; R: roots; S: stems.
Error: within repetition (pool of 3 plants, one from each block) variation (n=3).

Supplemental Table 4. List of selected genes/TC modulated in *lha-1* mutant and cited in the text.

Probe set ID	Transcript ID	Annotation	Fold change
		2,3-oxidosqualene cyclases	
Mtr.46511.1.S1_at	Medtr8g018540		2.1 down
Mtr.46511.1.S1_x_at			
Mtr.46512.1.S1_at	Medtr8g018550		1.8 down
Mtr.46514.1.S1_x_at	Medtr8g018610	Similar to *MtbAS*1	1.8 down
		Cytochrome P450s	
Mtr.8618.1.S1_at	TC100810	CYP93E2	2.3 down
Mtr.37299.1.S1_at	TC100323	CYP72A67	2.6 up
Mtr.37298.1.S1_at	TC100322	CYP72A68	1.6 up
Mtr.10812.1.S1_at	Medtr5g102050	CYP71D64	1.7 down
Mtr.6667.1.S1_at	Medtr8g050160	Putative CYP76C2	2.4 up
Mtr.10692.1.S1_at	TC107626	CYP76E1	1.6 up
Mtr.12208.1.S1_at	TC93934	CYP76X2	1.7 up
Mtr.6322.1.S1_s_at	BQ139200	Similar to CYP72A1	1.5 down
Mtr.43267.1.S1 at	Medtr8g045080	CYP72A65	1.8 down
Mtr.4947.1.S1_at	AW127462	CYP715A2	2.0 down
Mtr.20735.1.S1_at	Medtr8g090600	Similar to CYP90C1	1.7 up
Mtr.21518.1.S1_s_at	Medtr2g104500	CYP74B, HPL3	1.6 down
Mtr.9885.1.S1_at	Medtr2g104500	CYP74B, HPL3	1.5 down
Mtr.12632.1.S1_at	TC95424	CYP81E9 isoflavone3'-hydroxylase	2.0 up
Mtr.38274.1.S1_at	TC102428	CYP90B	1.9 up
Mtr.9112.1.S1_at	TC102429	CYP90B	1.8up
Mtr.42172.1.S1_s_at	TC110889	Similar to CYP707A3	1.5 up
		Glycosyltransferases	
Mtr.12473.1.S1_a	TC94916	UGT73F3	1.5 down
Mtr.11236.1.S1_at	Medtr2g008370	UGT91H5	2.7 down
Mtr.8530.1.S1_s_at	Medtr2g008360	UGT91H6	1.5 down
Mtr.49684.1.S1_at	Medtr7g076790	Similar to *Mt*UGT73F3	1.6 down
Mtr.43628.1.S1_at	Medtr5g076110	Similar to *Mt*UGT73F3	3.5 up
Mtr.37367.1.S1_at	Medtr2g008380	Similar to *Mt*UGT91H6	1.6 down
Mtr.39289.1.S1_at	TC104594	Putative GT	1.8 up
Mtr.3013.1.S1_at	Medtr6g014410	Putative GT	1.6 down
Mtr.42552.1.S1_at	TC111804	Putative GT	1.9 up
Mtr.40639.1.S1_at	TC107730	Putative GT	1.8 up
Mtr.36069.1.S1_s_at	AJ845941	Putative GT	1.5 down
Mtr.16941.1.S1_at	Medtr4g130290	Putative GT	2.0 down
Mtr.29993.1.S1_at	Medtr3g009370	Putative GT	1.7 up
Mtr.44439.1.S1_at	Medtr6g014590	Putative GT	1.7 up
Msa.2667.1.S1_at		Putative GT	1.5down
Msa.2806.1.S1_at		Putative GT	1.6 up
Msa.1673.1.S1_at		Putative GT	2.06up
Msa.1267.1.S1_at		Putative GT	1.8up
		Pathogenesis related genes	
Mtr.47298.1.S1_at		PGIP	5.4 down
Mtr.42876.1.S1_at		Similar to *Ps*PRP4	7.5 up
Mtr.41478.1.S1_at		Similar to *Mt*PR10-1 ABR18	5.0 up

Supplemental Table 5. List of primers used in this research.

Primer name	**Primer sequence (5' – 3')**
Right 1	CGTTCAAGATGCCTCTACCG
Left 1	TATCTTCCACACGTGAAAATGC
Right 2	AGACGTTCCAACCACGTCTT
Left 2	TTTTTATTTCATCCGACATGG
Primer RACE 3'	TATCTCTACCAATTGATTTGCCAGG
Primer RACE 5'	CCTGGCAAATCAATTGGTAGAGATA
Primer RACE 5'nest	ATCAATTGGTAGAGATATGATTCC
ATG2fw	ATGGAGCCTAATTTCTATCTC
TAA2rw	TTAAGCTTTGTGTGGATAAAG
Msc27fw	GATGAGCTTCTGTCAGACTC
Msc27rw	GCTACCATCATCATGCATGC
CYPfw	TTTGCCACCTGGTAAAATGG
CYPrw	AGCCTTTGTTGAATGGTGTTC
InsBfw	GCCAGTGAGGTTGGCTTAGT
InsBrw	TCTCAAGAGTGAGATATACCGG
PSKI	GTAAAACGACGGCCAGTGA
P450fw	GGAAATTGCAAAATCGAAACC
P450rw	AAAGAACCGAATGAAACCAATCA
CypEcoRIfw	GAATTCATGGAGCCTAATTTCTATCTCTCCC
CypClaIrev	ATCGATTTAGCTTTGTGTGGATAAAGGC
CYP-38FW	ACTGCCATTGCACCATTGTA
CYP-220RW	CCAGGTGGCAAATTTAATGG
Msc27 269FW	CACCCAAACTAGATGCAGAGAA
Msc27 424rev	CACCATCCTTGTAGTAGGCAAA