

Supplementary Information for

Root-specific camalexin biosynthesis controls the plant growth promoting effects of multiple bacterial strains

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Fig. S1. Natural variation in sulfatase activity

A Sulfatase activity in a typical tray. Plants were grown for 2 weeks in soil (10%)/sand mixture, afterwards 2 soil samples were taken per plant and sulfatase activity was measured. Data are presented as means and S.E. from 10 samples derived from 5 independent plants. Different letters mark statistically different values (one way ANOVA).
B Distribution of the sulfatase activity among Arabidopsis accessions. Sulfatase activity was determined in soil from 173 Arabidopsis accessions varieties. Shown is the frequency of the activity among the 173 accessions.

C Manhattan plot of GWAS results obtained by GWAPP. Plotted are scores as $-\log_{10}p$ for each marker on the 5 Arabidopsis chromosomes.



Fig. S2. Scheme of camalexin biosynthesis pathway.

Scheme adapted from Su et al. (2011). The possible position of the newly characterised CYP71A27 and CYP71A28 is indicated in red with a question mark.

	сур71А27	сур71А28	сур71А12	сур71А13
CYP71A12	9.9	5.02	0	5.33
CYP71A13	6.38	3.94	10	0
CYP71A27	0	0.7	1.05	0.49
CYP71A28	1.71	0	1.29	0.86
GST6	3.32	1.45	3.79	3.04
GGP	0.79	0.64	0.77	0.6
CYP71B15	0.94	0.56	1.03	0.83
МАМ	1.26	1.17	1.4	1.24
TSB	1	1	1	1.06
CYP79F1	0.28	0.39	1.44	0.05
СҮР79В2	0.56	0.56	0.65	0.32
MYB28	0.83	0.91	0.93	0.87
MYB51	1.43	0.89	1.2	0.94
SUR1	0.93	0.53	0.58	0.57
SOT16	1	0.61	0.64	0.63
SOT17	1	0.66	0.86	0.52

Fig. S3. Expression analysis of mutants in P-450 71A genes potentially involved in camalexin synthesis

RNA was isolated from roots of the 5 genotypes and accumulation of transcripts for genes of camalexin (black) and glucosinolate (red) synthesis was compared by qPCR. Data are shown relative to expression in Col-0, red boxes mark transcripts accumulating to a greater extent in the mutants and blue boxes indicate those that are repressed. The analysis was performed with three independent RNAs measured in duplicates.



Fig. S4. Glucosinolate accumulation is not affected by loss of CYP71A27 and CYP71A28

Col-0, *cyp71A27*, and *cyp71A28* plants were grown for 2.5 weeks on vertical agarose plates. Glucosinolate accumulation was determined in leaves by HPLC. Data are presented as means and S.D. from 4 biological replicates consisting of 2 shoots.



Fig. S5. CYP71A27 and CYP71A13 are important for plant microbe interaction.

A Col-0 and *cyp71A27* were grown for 2 weeks in presence of *Pseudomonas* sp. CH267, *P. simiae* WCS417r, *Paraburkholderia phytofirmans* PsJN, *Burkholderia glumae* PG1 or 10 μ M MgCl₂ as mock, and the fresh weight of the whole plants was measured. Data are presented as means and S.E. from at least 20 plants grown on 4 independent plates. Asterisks indicate significant differences between mock and bacterial treatment at p<0.05 (Student's T-test).

B Col-0, *cyp71A27*, and *cyp71A13* were grown for 2 weeks in presence of *Serendipita indica*, and the colonization rate was determined by quantification of fungal DNA in roots. Data are presented as relative abundance of fungal *SiTEF* transcripts compared to Arabidopsis *UBQ5* transcripts and as means and S.D. from 4 independent biological repetitions. The value in Col-0 was set to 1, asterisks indicate significant differences to Col-0 at p<0.05 (Student's T-test).



Fig. S6. Expression of *CYP71A27* and related CYP71 genes in different tissues.

The data was obtained from the Araport portal and show gene expression profiles in *Arabidopsis thaliana* developmental stages, organs and parts based on RNA-seq analysis. The transcript levels are shown in transcripts per million (TPM) and coloured according to log_2 TPM.



В

CH267

WCS417r



Fig. S7. CYP71A27 has a different expression pattern from CYP71A12

GUS stainings of transgenic plants expressing CYP71A12pro::GUS (21) (upper panel) and CYP71A27pro::GUS (bottom panel).

A Seven days old seedlings were transferred to liquid nutrient solution and treated with *Pseudomonas* sp. CH267 and *P. simiae* WCS417r (final OD_{600} =0.002), 1 µM flg22, or 10 µM MgCl₂ as mock for 24 hours before GUS staining. Scale bars, 0.2 mm. Photos representative of at least 7 plants. Bottom row: The distance between root tip to the beginning of GUS staining was measured in *CYP71A27pro::GUS* plants after the different treatments and compared to mock. Data are shown as means and S.D. from at least 7 plants.

Right column: Expression analysis of *CYP71A12* and *CYP71A27*. RNA was isolated from roots of the treated seedlings and accumulation of transcripts for *CYP71A12* and *CYP71A27* was compared by qPCR. The analysis was performed with three independent RNAs measured in duplicates, data are shown as means and S.D. expression in mock is set to 1.

Asterisks indicate significant differences to mock at p<0.05 (one way ANOVA).

B Five days old seedlings were transferred on plates containing *Pseudomonas* sp. *CH267* and *P. simiae WCS417r* (final OD_{600} =3.2 x 10⁻⁶) or 10 µM MgCl₂ and cultivated for 7 days. For flagellin treatment 2 week old plants were treated with 1 µM flg22 for 5 h. The plants were stained for GUS activity and photos were made with a Leica MZ 16 F stereomicroscope. Scale bars, 5 mm. Photos representative of at least 6 plants. Arrows point to flg22-induced expression of *CYP71A12* in root tips.



Fig. S8. CYP71A27 is not involved in pathogen defense in the leaves

Col-0, *cyp71A27*, and *cyp71A13* plants were grown for 4 weeks at short days and inoculated with pathogens.

Three leaves per each of 6 plants were inoculated with four 5 μ l droplets containing *Botrytis cinerea* B05.10 spores (5 x 10⁴/ml). After 3 days the lesion diameter (**A**) and area (**B**) were determined. Shown are means and S.E. from 72 lesion mesasurements.

C Leaves were inoculated with *Pseudomonas syringae pv maculicola* ES4326 (Psm) carrying the LUX operon from *P. luminescens.* The luminescence per area of leaf disks (RLU) was determined.

Camelexin was determined in leaves inoculated with *Botrytis* (**D**) or Psm (**E**) by HPLC. Data are presented as means and S.D. from 3 independent pools of 6 leaves (3 per plant).

F Col-0, *cyp71A27*, and *cyp71A13* plants were treated with $AgNO_3$ and camalexin was determined in the shoots and roots by HPLC. Data are shown as means and S.D. from three independent pools of at least 10 plants.

Asterisks indicate significant differences to Col-0 at p<0.05 (one way ANOVA).



Fig. S9. Sulfatase activity of CH267, MPI9 and MPI491 bacterial strains. Bacterial strains were cultivated on an M9 minimal medium using either $MgSO_4$ or p-nitrophenyl sulfate (PNPS) as the sole sulfur source. After 48 hours growth, bacterial sulfatase activity was quantified by spectrophotometric measurement of p-nitrophenyl (PNP) concentration in the culture supernatant at 400 nm. Shown are means and S.D. from four replicates.



Camalexin concentration (nM)

Fig. S10. Effect of camalexin on bacterial growth.

Growth of five bacterial strains was measured in the presence of different camalexin concentrations: 0, 3 nM, 10 nM, 25 nM, 75 nM, 1 μ M, 10 μ M, and 100 μ M. Bacteria were cultivated on ½ TSB medium in 48-well plates in a plate reader at 28°C for 24 h with shaking. For each well, OD600 was measured every 10 min without correcting for path length. Resulting growth curves were quantified by integrating the Area Under Curve (AUC), and AUC values for different camalexin concentrations are normalised versus the 0 camalexin control for that strain. Shown are means and S.D. from three replicates.



С

0.5

0.4

0.1

0

000 0.3 000 0.2



В





D PG1 MPI9 0.5 0.4 O Col-0 O Col-0 000 0.3 00 0.2 OCyp71A27 Cyp71A27 OCyp71A12 OCyp71A12 0.1 0 5 10 15 20 25 5 15 20 25 0 0 10 Time (h) Time (h) MPI491



Fig. S11. Growth of bacteria on root extracts.

Growth of five bacterial strains was measured in media where the sole carbon source was root extract harvested from different Arabidopsis genotypes. M9 minimal media were formulated to contain root extract as the sole carbon source, with a carbon concentration of 720 mg C/I. In a 48-well plate, washed bacterial cells were inoculated into growth media at an OD600 of 0.05, and cultivated in a plate reader at 28°C for 24 h with shaking. For each well, OD600 was measured every 10 min without correcting for path length.



Fig. S12. Quantification of bacteria in roots.

Col-0 and *cyp71A27* were grown for 2 weeks in presence Pseudomonas sp. CH267, MPI9, and MPI491, with or without addition of 10 μ M camalexin (cam). The bacterial titre was determined by colony counting. Data are presented as means and S.D. from 8 plates per genotype and treatment and the experiments was independently repeated. n.s. No significant differences for the individual strains were detected.



Fig. S13. Camalexin exudation

Three representative accessions of the two *CYP71A27* haplotypes were grown for 7 days on sterile 100 μ m nylon mesh (six seedlings per sample) in 12 well plates placed on 1 ml of ½ MS medium with 0.5 % sucrose. Seven μ l of *Pseudomonas* sp. CH267, *P. simiae* WCS417r cultures diluted to OD₆₀₀= 0.0001, or 10 μ M MgCl₂ as mock were inoculated into each well. The plates were further incubated under the same conditions for 6 days with occasional shaking. Camalexin was determined in the nutrient solution after purification on 1 ml solid phase extraction tubes (Discovery-DSC18) by HPLC.

Data are shown as means and S.D. from four exudate samples from 6 plants, normalized per FW of the whole seedlings. Two way ANOVA confirmed that the camalexin amount depends on haplotype ($P = 1.7 \times 10^{-5}$), treatment ($P = 8.2 \times 10^{-5}$) and haplotype:treatment (P = 00059). Different letters indicate significantly different values at p<0.05.



Camalexin concentration (nM)

Fig. S14. Effect of camalexin on bacterial sulfatase activity.

Bacterial strains were cultivated in a 48-well plate on an M9 minimal medium using pnitrophenyl sulfate (PNPS) as the sole sulfur source, in the presence of different camalexin concentrations: 0, 3 nM, 10 nM, 25 nM, 75 nM, 1 μ M, 10 μ M, and 100 μ M. (A) Extracellular PNP: After 48 hours growth, bacterial sulfatase activity was quantified by spectrophotometric measurement of p-nitrophenyl (PNP) concentration in the culture supernatant at 400 nm. Shown are means and S.D. from three replicates. (B) Growth rate: For each well, OD600 was measured every 10 min without correcting for path length. Resulting growth curves were fitted against the logistic growth equation, and shown here are the maximum growth rate (r) values across different camalexin concentrations. Values are normalised versus the 0 camalexin control for that strain. Shown are means and S.D. from three replicates.



Fig. S15. Complementation of *cyp71A27* mutant.

A Col-0, *cyp71A27*, and 6 complemented lines were grown for 2 weeks in soil (10%)/sand mixture, afterwards 2 soil samples were taken per plant and sulfatase activity was measured. Data are presented as means and S.E. from 10 samples corresponding to 5 independent plants. Different letters indicate differences significant at p<0.05 (one way ANOVA).

B Col-0, *cyp71A27*, and 6 complemented lines were grown for 2 weeks in presence of *Pseudomonas* sp. CH267 or 10 μ M MgCl₂ as mock, and the fresh weight of the whole plants was measured. Data are presented as means and S.E. from at least 20 plants grown on 4 independent plates. Asterisks indicate significant differences between mock and bacterial treatment at p<0.05 (Student's T-test).

C RNA was isolated from roots of the 8 genotypes and *CYP71A27* transcript accumulation was compared by qPCR. The value in Col-0 was set to 1. Data are shown as means and S.D. from three independent plants measured in duplicates.

Supplementary Table S1. Sulfatase activity in soil from 172 *Arabidopsis thaliana* accessions Sulfatase activity (nmol/min/g soil); shown are means from 10 soil samples, corresponding to 5 independent plants and activity relative to Col-0.

accession	ID	mean activity	activity rel. to Col-0
Aa-0	7000	96,24	1,13
Alc-0	6988	129,47	1,52
ALL1-2	1	157,67	1,85
ALL1-3	2	56,96	0,67
Alst1	6989	269,32	3,16
Amel-1	6990	95,14	1,12
An-1	6898	126,97	1,49
Ang-0	6992	110,68	1,30
Ann-1	6994	61,79	0,73
App1-16	5832	144,02	1,69
Baa-1	7002	104,24	1,22
Be-1	7011	90,02	1,06
Belmonte-4-94	957	127,29	1,49
Benk-1	7008	69,79	0,82
Bg-2	6709	188,98	2,22
Bil-5	6900	65,99	0,77
Blh-1	8265	98,13	1,15
Boot-1	7026	109,87	1,29
Bor-1	5837	108,17	1,27
Brö1-6	8231	220,44	2,59
Bs-2	7004	67,25	0,79
Bsch-0	7031	134,97	1,58
Bu-0	8271	86,39	1,01
Bu-8	7056	129,50	1,52
Bur-0	6905	118,23	1,39
C24	6906	103,50	1,22
Ca-0	7062	119,10	1,40
Can-0	8274	164,92	1,94
Cha-0	7069	141,05	1,66
CIBC-2	6727	168,85	1,98
CIBC-4	6729	191,32	2,25
Cit-0	7075	151,41	1,78
CLE-6	78	222,04	2,61
Co-2	7078	46,09	0,54
Co-4	7080	118,56	1,39
Col-0	6909	85,17	1,00
Com-1	7092	97,97	1,15
Cvi-0	6911	91,29	1,07
Da-0	7094	60,35	0,71
Di-1	7098	48,55	0,57
Do-0	7102	49,40	0,58
Dr-0	7106	168,03	1,97
Dra-0	7103	131,31	1,54
Dra3-1	8283	108,31	1,27

DralV.1.5	5887	126,94	1,49
Ede-1	7110	65,41	0,77
Eden-1	6009	81,76	0,96
Es-0	7126	38,25	0,45
Est-0	7128	124,67	1,46
Est-1	6916	89,20	1,05
Fäb-2	6917	113,59	1,33
Fei-0	8215	45,12	0,53
Fja1-1	8422	73,08	0,86
Fja1-5	6020	121,24	1,42
Fr-4	7135	171,26	2,01
Ga-2	7141	89,76	1,05
Ge-0	8297	132,54	1,56
Gel-1	7143	182,02	2,14
Gie-0	7147	81,07	0,95
Go-0	7151	123,34	1,45
Gr-1	8300	14,86	0,17
Gu-1	7150	111,53	1,31
Gul1-2	8234	149,34	1,75
Ha-0	7163	78,36	0,92
Hn-0	7165	157,45	1,85
Hod	8235	151,61	1,78
Hov-3-2	6036	112,61	1,32
Hovdala-2	6039	87,16	1,02
Hs-0	8310	18,95	0,22
HSm	8236	54,00	0,63
In-0	8311	70,53	0,83
JEA	91	69,68	0,82
Jm-1	7178	90,48	1,06
KBS-Mac-8	1716	86,58	1,02
Kin-0	6926	46,84	0,55
Kno-18	6928	130,41	1,53
Krot-0	7203	109,88	1,29
Krot-2	7205	127,00	1,49
Kulturen-1	8240	295,57	3,47
Kyoto	7207	60,68	0,71
LAC-3	94	91,98	1,08
LDV-25	116	161,90	1,90
Ler-1	6932	107,62	1,26
Li-7	7231	86,46	1,02
Lip-0	8325	326,20	3,83
LL-0	6933	217,31	2,55
Lm-2	8329	134,89	1,58
Lom1-1	6042	167,43	1,97
Lov-1	6043	133,17	1,56
Lov-5	6046	116,55	1,37
Mc-0	7252	98,82	1,16
Mh-0	7255	161,15	1,89
MIB-84	223	80,91	0,95

MNF-Che-2	1925	75,80	0,89
Mnz-0	7244	134,54	1,58
Mr-0	7522	76,79	0,90
Mrk-0	6937	113,89	1,34
Mv-0	7248	121,20	1,42
N13	7438	94,25	1,11
N7	7449	114,53	1,34
Nc-1	7430	101,44	1,19
NC-6	8246	85,26	1,00
No-0	7275	45,93	0,54
Nyl-13	9433	143,41	1,68
Nz-1	7263	126,05	1,48
Or-0	7282	99,34	1,17
Ors-2	7284	119,13	1,40
Oy-0	6946	245,67	2,88
PAR-4	259	60,64	0,71
Paw-3	2150	98,72	1,16
Pent-1	2187	14,36	0,17
Per-1	8354	84,32	0,99
PHW33	7504	57,34	0,67
PHW-34	7505	150,49	1,77
PHW35	7506	45,82	0,54
PHW36	7507	64,31	0,76
PHW37	7507	141,02	1,66
Pi-0	7298	45,34	0,53
Pla-0	7300	170,62	2,00
Pro-0	8213	40,69	0,48
Pt-0	7305	50,77	0,60
Pu2-23	6951	49,40	0,58
Rak-2	8365	127,45	1,50
Rd-0	8366	20,43	0,24
Ren-1	6959	231,48	2,72
Rev-2	6076	182,51	2,14
Rmx-A180	7525	95,25	1,12
Rou-0	7320	88,05	1,03
RRS-10	7515	147,84	1,74
Sav-0	7340	119,92	1,41
Sei-0	7333	76,65	0,90
Sg-1	7344	79,41	0,93
Shadahra	6962	48,96	0,57
St-0	8387	132,69	1,56
Ste-3	2290	54,27	0,64
T1040	6094	42,59	0,50
T1060	6096	91,25	1,07
T1110	6100	15,44	0,18
T540	6112	165,52	1,94
T620	6119	93,70	1,10
TDr-18	6203	118,30	1,39
Ting-1	7354	226,55	2,66

Tol-0	7356	181,41	2,13	
Tomegap-2	6242	163,28	1,92	
Tottarp-2	6243	101,35	1,19	
TOU-A1-62	328	153,77	1,81	
TOU-E-11	366	260,62	3,06	
TOU-H-13	374	132,01	1,55	
TOU-K-3	386	96,07	1,13	
Ts-1	6970	49,43	0,58	
Tscha-1	7372	72,66	0,85	
Tsu-0	7373	39,95	0,47	
Tul-0	7377	180,23	2,12	
Uk-2	7379	125,52	1,47	
UKNW06-436	5606	197,82	2,32	
UKSE-278	5122	101,52	1,19	
UII2-3	6973	88,56	1,04	
UII2-5	6974	40,09	0,47	
UII3-4	6413	84,88	1,00	
Utrecht	7382	54,82	0,64	
Van-0	7383	48,90	0,57	
Ven-1	7384	152,46	1,79	
VOU-1	390	33,31	0,39	
Wa-1	7394	60,68	0,71	
Wc-2	7405	60,16	0,71	
WI-0	7411	35,68	0,42	
Ws-0	6980	138,52	1,63	
Ws-2	6981	60,15	0,71	
X328PNA054	8692	133,55	1,57	
Yo-0	6983	144,79	1,70	
Zdr-6	6449	125,76	1,48	
Zu-1	7418	85,45	1,00	

Supplementary Table S2. Markers significantly associated with variation in sulfatase Shown are positions of markers with score $(-\log_{10}P)$ higher than 4.

chromosome	position	score	chromosome	position	score
5	16093269	5,781	2	8060333	4,266
2	8948991	5,768	4	7296652	4,266
5	18619037	5,387	1	898397	4,246
2	6300485	5,356	3	18002103	4,229
4	7369340	5,306	1	22960034	4,213
1	13052365	5,211	1	17864022	4,189
3	21352347	5,137	3	18002412	4,182
1	5484572	5,112	1	17600900	4,163
4	9865729	5,072	5	8429895	4,147
4	9874455	5,072	3	2366209	4,145
4	9885694	5,072	5	2685965	4,145
5	4734285	5,070	2	8612698	4,114
3	4559978	5,067	2	8583271	4,093
2	7332044	4,830	2	8586121	4,093
3	4574994	4,825	2	10501739	4,092
5	19728614	4,816	4	15210413	4,067
5	23670216	4,810	3	1145882	4,065
1	22744494	4,798	3	12599993	4,061
5	8427481	4,797	5	23700786	4,056
4	10930013	4,780	5	23705451	4,056
2	8588043	4,775	5	23715848	4,056
4	12026369	4,758	5	23777633	4,056
5	23765316	4,687	1	6895487	4,030
5	22814448	4,682	5	1557906	4,024
3	4555946	4,655	3	11052579	4,016
3	4556265	4,655			
2	8672858	4,611			
5	3912479	4,575			
3	8300660	4,529			
5	16463903	4,508			
5	18036619	4,441			
5	3373458	4,409			
1	17866252	4,407			
1	5062465	4,404			
5	18552017	4,386			
5	20299888	4,358			
4	7283307	4,339			
5	14734084	4,329			
2	272607	4,326			
1	2214123	4,322			
2	272690	4,315			
1	22744473	4,272			
3	12912061	4,272			
1	12073641	4,266			
1	12111200	4,266			

Supplementary Table S3. Candidate genes for analysis of T-DNA lines.

"gene" is the candidate gene selected within the linkage disequilibrium.

"p sulfatase" represents the p value of the comparison of sulfatase activity with Col-0. Red marks significant differences at p<0.05

rank	chrom.	position	score	gene		annotation	T-DNA line	p sulfatase	sulfatase activity relative to Col-0
2	2	8948991	5,768113	At2g20780	MRS	Major facilitator superfamily protein	SAIL_759_E05	0,35	0,9
3	5	18619037	5,386588	At5g45875		family of small, secreted, cysteine rich proteins	SALK_089063	0,14	0,72
4	2	6300485	5,356145	At2g14750	APK1	APS kinase 1	SALK_053427	0,41	1,06
7	3	21352347	5,13653	At3g57650	LPAT2	lysophospatidyl transferase			
8	1	5484572	5,112086	At1g15950	CCR1	cinnamoyl CoA reductase	GK-622C01	0,007	0,46
13	3	4559978	5,066509	At3g13870	RHD3	root hair defective			
18	1	22744494	4,797908	At1g61680	TPS14	terpene synthase			
20	4	10930013	4,77969	At4g20235	CYP71A28	cytochrome P-450	SALK_064792C	0,04	0,49
				At4g20240	CYP71A27	cytochrome P-450	SALK_053817C	0,04	0,63
21	2	8588043	4,775429	At2g19880	GCS	Glucosylceramide synthase			
24	5	22814448	4,681923	At5g56360	PSL4	defense response to bacterium,	SALK_039458C	0,27	0,87
29	3	8300660	4,529327	At3g23240	ERF1	ethylene response factor			
31	5	18036619	4,440517	At5g44620	CYP706A3	cytochrome P-450			
				At5g44630		Sesquiterpene synthase	SALK_151777C	0,33	0,92
33	1	17866252	4,406965	At1g48370	YSL8	metal-nicotianamine transporter	SALK_076262_10	0,03	0,54
				At1g48380	RHL1	ROOT HAIRLESS 1			
34	1	5062465	4,403505	At1g14700	PAP3	purple acid phosphatase 3	SALK_081178	0,04	0,67
49	3	18002103	4,228665	At3g48560	AHAS	Acetohydroxy Acid Synthetase			
50	1	22960034	4,21292	At1g62180	APR2	APS reductase 2	GABI_108G02	0,16	1
54	5	8429895	4,146978	At5g24655	LSU2	Low Sulfur 2	SALK_069114	0,04	0,73
				At5g24660	LSU4	Low Sulfur 4	SALK_031648C	1,17	
59	2	8586121	4,092993	At2g19860	HXK2	hexokinase 2			
68	1	6895487	4,029585	At1g19920	ATPS2	ATP sulfurylase 2	SAIL775D12	0,49	1
69	5	1557906	4,024213	At5g05260	CYP79A2	cytochrome P-450	GK-913H04	0,13	0,74

annotation gene At2g20780 Major facilitator superfamily protein At5g45875 family of small, secreted, cysteine rich proteins At2g14750 APS kinase 1 At1g15950 cinnamoyl CoA reductase At4g20235 cytochrome P-450 At4g20240 cytochrome P-450 At5g56360 defense response to bacterium, At5g44630 Sesquiterpene synthase At1g48370 metal-nicotianamine transporter At1g14700 purple acid phosphatase 3 At1g62180 APS reductase 2 At5g24655 Low Sulfur 2 At5g24660 Low Sulfur 4 At1g19920 ATP sulfurylase 2 At5g05260 cytochrome P-450

homozygous T-DNA line SAIL 759 E05 SALK 089063 SALK 053427 GK-622C01 SALK 064792C SALK 053817C SALK 039458C SALK 151777C SALK 076262 10 SALK 081178 GABI_108G02 SALK 069114 SALK 031648C SAIL775D12 GK-913H04

SALK LB primer GABI LB primer

primers for verification of T-DNA insertion gcgatgtagacaggagcg ttagcaaggagaatatggaaa gctttcgatggcttcttc gtcgacgtagcctcacc cctgccgaggagatgg ttccaatcttctccgtagg ccttcagctgctggctc gggacttggagagattgg gccatcgaatagcagacc ctaatctctcaatggctacc gtgctcttaagggtcttaaa gatccaacaatctaagaacc agccgataagaggatacatc tctcaatttggtctataaacg ttcgtccgacagagtagg

attttgccgatttcggaac atattgaccatcatactcattgc gaatcttccggaggttgg atgaagtctactaccttgttc gacccaaatcacacatcc cttgaccttggcctcagc gcttcaccactttcttagc gtctccttcccaagcgag aattgagaaggtagaggag tctacgagcaaaagcctc tagcgacagctatgaacac aaggtttctgcaagctcg ctcacacccgattgacac gaaaaggaggaaactatgtg ttagatcaaagtaaaatggtcc ttaaaggactagcccaacc cattaactatgaagcgtaagg Supplementary Table S5. Bacterial strains used.

	NCBI	antibiotics	sulfatase	ref.
	Taxonomy	resistance	activity	
	ID			
Pseudomonas sp. CH267	1634009	ampicillin	+	(26)
Pseudomonas simiae WCS417r		rifampicin	-	(27)
Paraburkholderia phytofirmans PsJN	398527	gentamicin	-	(28)
Pseudomonas sp. Root9 (MPI9)	1736604		+	(4)
Rhizobium sp. Root491 (MPI491)	1736548		-	(4)
Burkholderia glumae PG1	595500	chloramphenicol	+	(29)

The NCBI Taxonomy number is included when available. Presence of sulfatase activity determined experimentally as production of p-nitrophenyl during growth on p-nitrophenyl sulfate.

Supplementary Table S6. Oligonucleotide primers used

qPCR					
TIP41	AT4G34270	TIP41-F	gaactggctgacaatggagtg	TIP41-R	atcaactctcagccaaaatcg
CYP71A12	AT2G30750	CYP71A12-F	tgtggtgtttggtccctatg	CYP71A12-R	ttgttcgtgagcagattgaga
CYP71A13	AT2G30770	CYP71A13-F	gatgttgtgtttgctccctatg	CYP71A13-R	ttgttggtgagcagattgaga
CYP71A27	AT4G20240	CYP71A27-F	ccctacggagaagattggaa	CYP71A27-R	ccagcttctctgtcattactttga
CYP71A28	AT4G20235	CYP71A28-F	ttctcctcctacggcgaata	CYP71A28-R	gaggagatggacagtgcataaa
GST6	AT2G47730	GST6-F	aagcaagaggcccacctt	GST6-R	tcttgactcgaaaagcgtca
GGP1	AT4G30530	GGP1-F	tgtcaagaaaattgatgagatgaag	GGP1-R	ccccttaccctggctatgat
CYP71B15	AT3G26830	CYP71B15-F	caccactgatcatctcaaagga	CYP71B15-R	cggtcattccccatagtgtt
MAM	At5g23020	MAM-F	ccgtgaacagtgttaagtacgc	MAM-R	cacccgcttttatcgattct
TSB	At5g54810	TSB-F	acgaagaagcgttggaagc	TSB-R	ggtaagctagtgcgtgtgagg
CYP79F1	AT1G16410	CYP79F1-F	agaccgatcttgccagctt	CYP79F1-R	ggtagattgccgaggatgg
CYP79B2	AT4G39950	CYP79B2-F	tgacggatcccaacaaaaag	CYP79B2-R	atgatcggccatcctgtg
MYB28	AT5G61420	MYB28-F	tggaccaactaccttaaacctga	MYB28-R	tctcgctatgaccgaccact
MYB51	AT1G18570	MYB51-F	ggccaattatcttagacctgaca	MYB51-R	ccacgagctatagcagaccatt
SUR1	At2g20610	SUR1-F	ggattctccccgcaagac	SUR1-R	catccagcggtcagaaaa
SOT16	At1g74100	SOT16-F	cgaagtcgtcgaactcacagagtt	SOT16-R	aaagaccttcgaggagacattcttg
SOT17	At1g18590	SOT17-F	ggaatccaaaaccataaacgacg	SOT17-R	cggatcttttggtctccagcc
UBQ5	At3g62250	AtUBI_F	ccaagccgaagaagatcaag	AtUBI_R	actccttcctcaaacgatga
PiTEF		03008_QPCR_F	gcaagttctccgagctcatc	03008_QPCR_R	ccaagtggtgggtactcgtt

cloning

cacctttgacctatactttactacc
attgtgcttatttaggaatgg
caccctatttaagtatttttattggac
tcacaaggttggctagtgc