Arabidopsis mutants impaired in glutathione biosynthesis exhibit higher sensitivity towards the glucosinolate hydrolysis product allyl-isothiocyanate

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Supplementary Figure S1. The ratio between the mean root length under a given AITC concentration and that of the control treatment calculated for 10 day old seedlings of the Col-0 WT and the glutathione mutants (A) *cad2-1*, (B) *zir1*, (C) *nrc1*, (D) *nrc2*.



Supplementary Figure S2. Root elongation analysis over time using WT and glutathione mutants grown either on control medium or on medium supplemented with 300 μ M AITC: (A) *pad2-1*, (B) *cad2-1*, (C) *zir1*, (D) *nrc1* and (E) *nrc2*. Root lengths were measured (n= 48 for mutants; n= 96 for WT) at each time point (at day 4, 7 and 10) and the mean values were used to calculate the average root length increment. Note that the values for WT are identical for each of the panels as all seedlings were grown in parallel.



Supplementary Figure S3. The ratio between the mean biomass under a given AITC concentration and that of the control treatment calculated for 10 day old seedlings of the Col-0 WT and the glutathione mutants (A) *cad2-1*, (B) *zir1*, (C) *nrc1*, (D) *nrc2*.



Supplementary Figure S4. Ratios of (A) mean root length and (B) mean biomass between a given treatment (AITC, BSO or AITC+BSO) and that of the control treatment calculated for 10 day old seedlings of the Col-0 WT and the glutathione mutant p*ad2-1*.



Supplementary Figure S5. Genotyping results of mutants used in this study (see also Supplementray Table S1) performed as described in more detail in the Methods section using the primers listed in Supplementary Table S2.

GSH1	20 49 49 49 50 100 120 140 140 150 200 1 A 1900 CONTENTIAL CONTEN	: 200
pad2-1 cad2-1		1 1
zirl		1 1
GSH1	220 220 240 240 260 280 300 400 400 400 400 400 400 400 400 40	: 400
pad2-1 cad2-1		1 1
zirl		1 1
GSH1	40 40 50 50 50 50 50 50 50 50 50 50 50 50 50	: 600
pad2-1 cad2-1		: :
zirl nrcl		1 1
GSH1	420 640 640 640 700	: 800
pad2-1 cad2-1		1 1
zirl nrcl		1 1
GSH1	200 240 260 260 260 260 260 260 260 260 260 26	: 1000
pad2-1 cad2-1		1 1
zirl nrcl	CAAGGATITEACT000007CAAGTC0ACTTAT0010CACCTCT00AGCTTT0CATCAAATT0CCT0010CT0AATTCAAAGTAATTATCAC001AGTAATTAT0CCAATGCCTCACCCTAAAACTAAACCAAATAACCAAATAACCAACTACCACC	: 178
GSH1	$000 \pm 1000 \pm 10000\pm 10000\pm 1000\pm 1000$: 1200
pad2-1 cad2-1	80480847474CCCA140CCA34801474CACCT11CC14474719480419480410474074110111C14481474748441141CC0481C14840420110004114111C1	: 131
nrcl	COTABALIC-COTTOCTGARGIAATOSGIATTOSTITCTIASIAATISCTCCRGT	: 378
GSH1		: 1400
pad2-1 cad2-1	ROSTOTITOTITANOTITICCTATORCORDICASORATICARCATAGTOTAROTOTICCTOTICASORATICORCATIONALIATICTICCTATORCORDINALIATICTICALIATICALIATICTICALIATICCTATORCORDINALIATICTICALIATICALIATICTICALIATICCTATORCORDINALIATICTICALIATICALIATICCIALIATICTIC	: 325
zirl nrcl	TOGIGI TI OTOTI AAOTI TI CCTATGACGAGTAAOATI TUACATAGTOTIAATGTOTI TCTOTOCOGGAGATACGACATTATGAGAA	: 473
GSH1	1420 1440 1460 1460 1460 1560 1560 1560 1560 1560 1560 1560 15	: 1600
pad2-1 cad2-1	TOTATION CONTINUES AND	: 98 : 469
zirl nrcl		1 1
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pad2-1 cad2-1	CTAACCATIGCCTCACTATTATCTAAATACAGATAGCAACGACGGACGCACTATTGCGAAATCCCCATTTACAGAAGGAAAGCCAAACGGATTCCTO	: 298
zirl		1 1
	• 1820 • 1840 • 1860 • 1880 • 1900 • 1920 • 1940 • 1960 • 1980 • 2000	
GSH1	· ACAGACACTGACAAGGACCGCACAGGAATGCTACCATTTGTTTTCGATGACTCTTTTGGATGAGTATCGATGTTTAGGTATGACAATTACATTAGCATTAGGTATGGACATGGCTATCGAGAACCACAGACCTCCTCAAAAACCAATGACTCTTGATGAGTATTGGAAAATTGATATGTGCTATTGTGGATT	: 2000
pad2-1 cad2-1	MCMCRCFGMCAMOGACCCACAGGANGCTACCATTTGTTTTGGATGACTCTTTTG90TGMGTATCGATGTTTAATCTATGGAAAAACAATTACATTGGTTGG	: 2000 : 308 : -
GSH1 pad2-1 cad2-1 zir1 nrc1	MCMCMCCGCCACAGGANGCTACCATTIGTTITICGATGACTCTTTT000TGAGTATCGATGTTTAATCTATGAAAAACAATTACATTGGTT00TTT00T	: 2000 : 308 : - : -
GSH1 pad2-1 cad2-1 zir1 nrc1	ACARACKTERCEMORYCCCCCLORAMATCTATCCATTECTTTEODTORATTATCTTTATCTATERABALACEMTIRCATERCENTEGTATERABALACCMTERCENTECTCAAAAACCMTERCENTETCTCTECTCOCCATECTCTERCENTETTTETCENTERCENTETTTETCENTERCENTETTTETCENTERCENTETTTETCENTERCENTETTTETCENTERCENTETTTETCENTERCENTETTTETCENTERCENTETTTETCENTERCENTETTTETCENTERCENTETTTETCENTERCENTETTTETCENTERCENTETTTETCENTERCENTETTTETCENTERCENTETT	: 2000 : 308 : - : -
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GSH1 pad2-1 zir1 nrc1 GSH1 pad2-1 cad2-1 zir1 nrc1		: 2000 : 308 : - : - : - : - : - : - : 15 : -
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GSH1 pad2-1 zir1 nrc1 GSH1 pad2-1 cad2-1 zir1 nrc1 g3H1 pad2-1 cad2-1 zir1 nrc1 g3H1 pad2-1 cad2-1		: 2000 : 308 : : : : : : : : -
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0381 pad2-1 rec1 0581 pad2-1 0581 pad2-1 rec1 0581 rec1 0581 rec1 0581 rec1 0581 rec1 0581 rec1 rec1 0581 rec1 re		: 2000 : 308 : : - : - : - : - : - : - : - : - :
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03811-1 9642-1 96642-1 96642-1 9664-1		: 2000 : 308 :

(A) GSH1 deficient mutants pad2-1, cad2-1, zir1 and nrc1

(B) GSH2 deficient mutant nrc2

GSH2 : nrc2 :	20 40 40 60 80 100 120 140 ArgggcAgtggctggctgttcttttctcttcttcttctctcttgcAatgccActgfttttctctattttttttctctatcttcttcttctcggeAAtgccAatggcttccttttcccAatgAgcttccttttcccAatgAgcttcgttttcttgag	SAAAT :	150
GSH2 : nrc2 :	160 • 180 • 200 • 220 • 240 • 260 • 280 • : CAGICACCTITICAGGIGIGGGAGATCATITAAGAIGAAAACCCATITIGGAGAAATTAGAIGAIGAGIIIGTICAGAAACTGGITTACGAIGCCCCCGTIGGGCTICCCTCACGGACCGGCGGGGG :	300 ACAAA :	300
GSH2 : nrc2 :	 320 * 340 * 360 * 380 * 400 * 420 * 440 AGTIAICAGGITIACTIACTIGECCACCACCACTAAAGTICGTOTTIGATETTGTGTTETGATAAAGTIAGAGAAAAATICGAAATTIATTATETTTATGAACCTTGTATAGCTGAATCATCATTATTICTTCGTCCTGGATTGTGTT . 	ragit :	450 -
GSH2 : nrc2 :	460 480 \$20 \$40 \$60 \$80 * rcgtigicitatagctitcgatgccatgitigatcigaatccaaagctitggtitiggtitiggtitiggtitiggatgaatcaggaaaggcaggatggatggatggatggatg	600 ATTCC :	600 -
GSH2 : nrc2 :	620 • 640 • 660 • 680 • 700 • 720 • 740 : CAGAAGCITAGIGAAGCIAGCIAGGIAATGITACCCCCTTTTCAATGAATTGATGGTGGGTGGGTGGGGGGAAATTCTTACAGGATAGTCTCTCTAGGTTGGTATCGCAATCTATCT	AACAA :	750
GSH2 : nrc2 :	760 * 780 * 820 * 840 * 860 * 880 * : GCGTAATGATCTITIGATIGATGTTCTAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGA	900 CTTGA :	900 -
GSH2 : nrc2 :	* 920 * 940 * 960 * 980 * 1000 * 1020 * 1040 : IICITIGGIICIGGAIGIIGIICATIIGGIIGTGIITGIGAICGCAGGACATICGIIIGGGIITACACCCGGIIIGAITATAIGCIIGACGAAGAAAAATICACTICICAGATGAAGAAAATACTAICICGIGIICGIIICCI :	rggcc :	1050
GSH2 : nrc2 :	1060 • 1080 • 1100 • 1120 • 1140 • 1160 • 1180 • : TIAGTEGETETTAGCCAGETACATCAGETATGEACAGEACCATETICEGEATTACCTGETETETITITIATACAGEGGAATTITATICCACTIGETEGEAGGETEATGECTEGATGEATAGEGGATCAGATTGGETA :	1200 FAGAC :	1200
GSH2 : nrc2 :	* 1220 * 1240 * 1260 * 1280 * 1300 * 1320 * 1340 : TCTGRARCGTGTACCTATARCCACTCCACATCCAATTTGCTGATGCATTGGCTARGCCTGGTGGAGTACACTAACCCAAGGTTAGCCTGGARCACATAATCAAGAA	AGAGT :	1350 -
GSH2 : nrc2 :	1360 * 1380 * 1400 * 1420 * 1440 * 1460 * 1480 * : TITICIIGGITICAGAGCGGIAGICAIGGIAATIGIACAGCAAGAAGACGCAACAIGIACGATCAACAITIGCIGAGCAGIATAITGAGAGAAAAATAICCICTITIGICIIGITICCIGGITICCAGGGGGICGATCIIGACI :	1500 ICTTA :	1500
GSH2 : nrc2 :	* 1520 * 1540 * 1560 * 1580 * 1600 * 1620 * 1640 : TGCTATATTTTGTACCACAAAAAATGGTGTTGTTCTTCCTTAGGTTCAGAAACACGGATAATATAGTTGTCATCAGGAAGACCCTTAGCAGAAGAGGAGGAAGAGCGATGAAAAGGAGGATGAAAACCCTTATGTGTGTAAGCATTTG :	STTTG :	1650
GSH2 : nrc2 :	1660 * 1680 * 1700 * 1720 * 1740 * 1760 * 1780 * : CATEGEGETITITEGEGAACTECCECTCATCTEGAAGGCTATETTAAGAGETITECATEGEGEGEGCGAAGCAGECGAGEGGETITATITECAGATCTGGECTATACTCCCETCAATGATCATCCGECTGAATCAGEGETAACTECGECAATGATCATCCGECTAACTECGECAATGATCATCCGECTAACTECGECAATGATCATCCGECTAACTECGECAATGATCATCCGECTAACTECGECAATGATCATCCGECTAACTECGECAATGATCATCCGECTAACTECGECAATGATCATCCGECTAACTECGECAATGATCATCCGECTAACTECGECAATGATCATCCGECTAACTECGECAATGATCATCCGECTAACTECGECAATGATCATCCGECTAACTECGECAATGATCATCGECAATGATCATCGECAATGATCATCGECAATGATCCATGATCATCGECAATGATCATGATGATCATCGECAATGATCATCGECAATGATCATGATGATCATCGECAATGATCATCGECAATGATCATGATGATCATCGECAATGATCATCGECAATGATCATCGECAATGATCATCGECAATGATCATCGECAATGATCATGATGATCATCGECAATGATCATCGECAATGATGATCATCGECAATGATCATGATGATCATGATCATGATGATCATGATGATCATGATGATCATGATGATCATGATGATCATGATGATGATGATGATGATGATGATGATGATGATGATGA	1800 CCGTC :	1800
GSH2 : nrc2 :	* 1820 * 1840 * 1860 * 1880 * 1900 * 1920 * 1940 * ARGITICCARTATIGIGGGETTATIGIGGARARICIGARICATATCATIGGITIATIGGTICARTIGCAGGARIGGARIGGARIGGARIGGTIATGAGGAGICTICAGGIGGARARGCCGAGGARIGGTIACCATTRAG :	CTGGC : CTGGC :	1950 67
GSH2 : nrc2 :	1960 * 1980 * 2000 * 2020 * 2040 * 2060 * 2080 * : ТССАЛБЛАЛТССАБСАЛБЛАСТВОСТАЛАССАБОТОТСТСБАБАВОТАЛАТБОАБОССОТТССАЛАТАТТСАЛАВОССАЛАЛАСТИТИТАЛСТГАЛАЛСАТВАБАЛАСАВОТИСТВОАСАЛСАЛАВА : ТССАЛБЛАЛТССАБСАЛБЛАСТВОСТАЛАССАБОТОТСТСБАБАВОТАЛАТБОАБОССОТТССАЛАТАТИТСАЛАВОССАЛАЛСТИТИТАЛСТГАЛАЛСАВОЛАСАВОТИСТВОАСАЛСАЛАЛАВА	2100 SGACA : SGACA :	2100 217
GSH2 : nrc2 :	2120 2140 2160 2180 2220 2240 : TIGCTAAGCIGAGGAAATGCTITGTGGGGCTITGGAGGACTGGATGACTCAGAAATIGTCAAGCAGGGCTATCGAAAAACCCGGATTGTITGTTATGAAGCCTCAGAGAGAAGGCGGAGGTTAGAACCCAATIGAGTCCCCCAATAG : TIGCTAAGCIGAGGAAATGCTITGTGGGGCTTGGAGCTGGGATGACTCAGAAATIGTCAAGCAGGCTATGGAAACCCGGATTGTITGTTATGAAGCCTCAGAGAGAAGGCGGAGGTTAGGACCCAATIGAGTCCCCCAATAG	- CTTCA : CTTCA :	2250 367
GSH2 : nrc2 :	2260 * 2280 * 2300 * 2320 * 2340 * 2360 * 2380 * : ACTITICGGGTCATATICTAGGCTGTGTTGTTGGATTGTTCCAGGAAACATCTATGGAGGAGAGGAGGGGAGGGA	2400 CCCAA :	2400 443
GSH2 : nrc2 :	* 2420 * 2440 * 2460 * 2480 * 2500 * 2520 * 2540 * ARGICICAMACHIGITICIGGIGCGAGAAGGCCTITACCATAAGCATCAGGACIGGAGAACICGGIGCTTACCICAGGIACAGAGAGAGAGAGAGAGAGAGAGAGAGAGA	STGCC :	2550
GSH2 : nrc2 :	2560 * 2580 * 2600 * 2620 * 2640 * 2660 * 2680 *	2700 GATTT :	2700
	1	:	-

(C) ggp1-1 (GK-319F10)



Size (bp)

1500

(E) **ggt2-1** (SAIL_6_G02) T-DNA G

Y

<u>M</u> I. II. III. WT M <u>M</u> I. II. III. WT M

Gene

(D) ggt1-1 (SAIL_1178_C01)





(G) ggt4 (GT_5_57895)

(F) ggt3 (ET1113)



(H) *mrp1* (SALK_017431)









(K) *opt1-3* (SALK_052985)



(L) opt6 (SALK_201534)





(O) *sultr4;2* (SALK_103827)



Supplementary Table S1. List of mutants used in our study. Mutants that are underlined in the table showed an increased susceptibility to the AITC treatment.

Mutant name	Mutant ID	Source/ Stock ID	Gene name	Gene ID	Description	Reference
<u>cad2-1</u>		Cobbett	GSH1/CAD2	AT4G23100	<i>cadmium sensitive</i> 2 ; γ -ECS/GSH1 is the first and rate- limiting enzyme of glutathione biosynthesis	1
clt1clt2clt3		Cobbett	CLT1 CLT2 CLT3	AT5G19380 AT4G24460 AT5G12170	<i>chloroquine-resistance transporter-like transporters 1, 2,</i> <i>3</i> ; transporters involved in glutathione transport from plastid to cytosol	2
ggp1-1	GK-319F10	N430598	GGP1	AT4G30530	γ -glutamyl peptidase 1 ; GGP1 hydrolyses γ -glutamyl peptide bonds	3
ggt1-1	SAIL_1178_C01	Ren	GGT1	AT4G39640	γ -glutamyl transpeptidase 1; GGT1 plays a role in the degradation of glutathione and its S-conjugates	4
ggt2-1	SAIL-6-G02	N862221	GGT2	AT4G39650	γ -glutamyl transpeptidase 2; GGT2 is involved in the degradation of glutathione and its S-conjugates	5
ggt3	ET1113	N26010	GGT3	AT1G69820	<i>γ-glutamyl transpeptidase 3</i> ; GGT3 plays role in the degradation of glutathione and its S-conjugates	6
ggt4	GT_5_57895	Grill	GGT4	AT4G29210	<i>γ-glutamyl transpeptidase 4</i> ; GGT4 is involved in the degradation of glutathione and its S-conjugates	7
mrp1	SALK_017431	N685353	MRP1/ABCC1	AT1G30400	multidrug resistance-associated protein 1 ; glutathione S-conjugate pump	8
mrp2-2	SALK_127425	N627425	MRP2/ABCC2	AT2G34660	<i>multidrug resistance-associated protein 2</i> ; glutathione S-conjugate pump	9
mrp3	SALK_044022	N662277	MRP3/ABCC3	AT3G13080	<i>multidrug resistance-associated protein 3</i> ; glutathione S- conjugate and chlorophyll catabolite transporter	10
<u>nrc1</u>		Schroeder	GSH1	AT4G23100	<i>non response to cadmium 1</i> ; γ -ECS/GSH1 is the first and rate-limiting enzyme of glutathione biosynthesis	11
<u>nrc2</u>		Schroeder	GSH2	AT5G27380	<i>non response to cadmium 2</i> ; glutathione synthetase is the second enzyme of glutathione biosynthesis	11

opt1-3	SALK_052985	N662479	OPT1	AT5G55930	oligopeptide transporter 1	12
opt6	SALK_201534	N689065	OPT6	AT4G27730	<i>oligopeptide transporter</i> 6 ; involved in the transport of glutathione derivatives	13
<u>pad2-1</u>		N3804	GSH1	AT4G23100	<i>phytoalexin deficient</i> 2 ; γ -ECS/GSH1 is the first and rate- limiting enzyme of glutathione biosynthesis	14
pcs1pcs2		Grill	PCS1 PCS2	AT5G44070 AT1G03980	<i>phytochelatin synthase 1 and 2</i> ; synthesis of phytochelatins and catabolism of glutathione conjugates	15
sultr1;2	SM_3.30250	N116961	SULTR1;2/SEL 1	AT1G78000	sulfate transporter 1;2; high-affinity sulfate transporter	16
sultr4;1	SALK_103873	N673083	SULTR4;1	AT5G13550	sulfate transporter 4;1; low-affinity sulfate transporter	17
sultr4;2	SALK_103827	N676417	SULTR4;2	AT3G12520	sulfate transporter 4;2; low-affinity sulfate transporter	18
<u>zir1</u>		Yeh	GSH1	AT4G23100	<i>zinc tolerance induced by iron 1</i> ; ECS/GSH1 is the first and rate-limiting enzyme of glutathione biosynthesis	19

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Supplementary Table S2. List of primers used for validation of the mutant lines tested in our study. (For: forward gene specific primer, Rev: reverse gene specific primer; T-DNA: primer annealing on T-DNA)

Mutant name	Primer sequence (5'-3')
_	For GGAGGATATACCCATCATGC
cad2-1	Rev GGTTGTAAAGCAAGACCAGC
	For AGAATGATGATTGGATCCTTAAGC
qqp1-1	Rev GATCGCTATGCTATCAGGAATCTC
551	T-DNA ATAATAACGCTGCGGACATCTACATTTT
	For GACTTGGCCTCAACGTATACATC
ggt1-1	Rev CGCGAGACTTAACAATACTTTGC
55	T-DNA TAGCATCTGAATTTCATAACCAATCTCGATACAC
	For CTCCCACCATTGTACTCAAGG
ggt2-1	Rev CTAGAGGCGGAGTATCCAAGG
	T-DNA TAGCATCTGAATTTCATAACCAATCTCGATACAC
	For GTATCACTTCTTGGTCACACTGC
ggt3	Rev GATCAAATAACTTACCTGCCACC
	T-DNA CCGTTTTGTATATCCCGTTTCCGT
	For AATCGGTGGTGGTTCTTTCTTGATT
ggt4	Rev TGTTGAGAAATCATCCATCTCGTTG
	T-DNA ACCCGACCGGATCGTATCGGT
	For ATTGGGGGTTTTATGTGAAGC
mrpl	Rev ACTTCACTGTATCCATTGCCG
_	T-DNA TGGTTCACGTAGTGGGCCATCG
	For GCTTTTCTGCGTTTGTAGTGG
mrp2-2	Rev TTTTCCCAAGCATAACACCTG
_	T-DNA TGGTTCACGTAGTGGGCCATCG
	For AAGTCAGCAATTGCTTTGGAG
mrp3	Rev AGGGAGCCTTTTTATGCTCAC
	T-DNA TGGTTCACGTAGTGGGCCATCG
	For CAAAGCATTTCACTTGAACC
nrei	Rev TTCTCATAATGTCGTATCTCCC
nral	For TGCTAGGCTGCTTATTGAGG
111.02	Rev TCCCTCACATCATCTCCATAG
	For AAAACCAAAGAAAACGGATCC
opt1-3	Rev GACTATTACCCACAACAACAAAATG
	T-DNA TGGTTCACGTAGTGGGCCATCG
	For AAAATTCAAACATTACAAGCCATG
opt6	Rev GATAGGGAAGGTCTTGGCATC
	T-DNA TGGTTCACGTAGTGGGCCATCG
nad2-1	For TGATATGATCAGGAAGTTTCG
puuz 1	Rev CAGTGTCTGTCCATATGTGG
	For _{pcs1} CGAATCCACTAACGAATCTTCC
	Rev _{pcs1} TTGATTCATCAAACCACCTCC
pcs1pcs2	For _{pcs2} GGTTCACGAATTATTGATAGCC
	Rev _{pcs2} CTCTGTTCTTCGAATCTCAGC
	T-DNA ATGGCGAAATCAAGGCATC
_	For ATTGGTGCATCATTCTTGACC
sultr1;2	Rev TCCGAGAACTGCAGTTCTAGG
	T-DNA CTTATTTCAGTAAGAGTGTGGGGGTTTTGG
_	For AGCTAAGACTGGCCTATCAGG
sultr4;1	Rev CGTTTTGGTGACTCTTGCTC
	T-DNA TGGTTCACGTAGTGGGCCATCG
1	For CCAGCAGCATGTATCGGTATC
sultr4;2	Rev ACCGTATATTGGTTGAAGCCC
	T-DNA TGGTTCACGTAGTGGGCCATCG
zir1	For CTGTACTGGAATGACATTTCGG
	Rev AGTCAGCTGTCAGATCCAGG