

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

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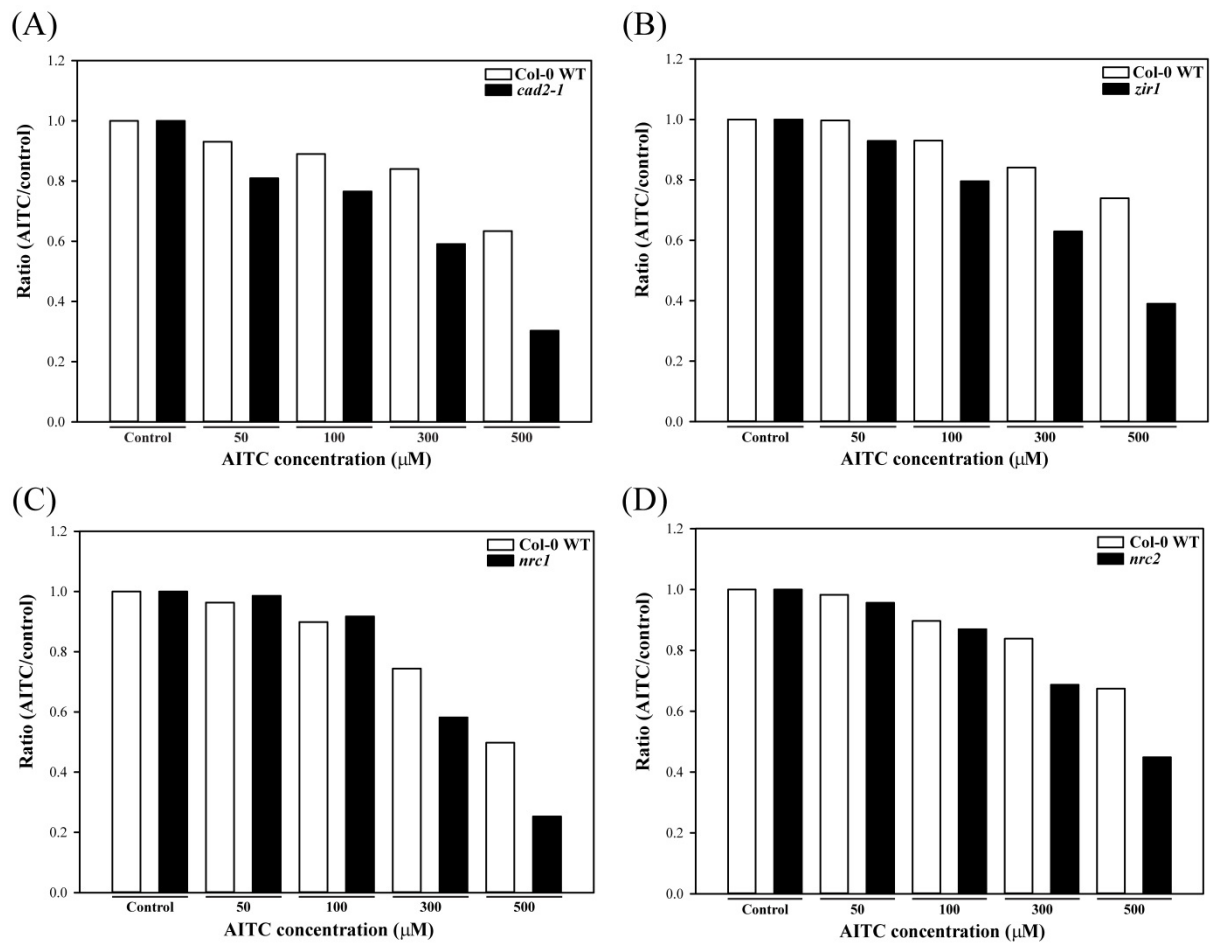
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Ralph Kissen

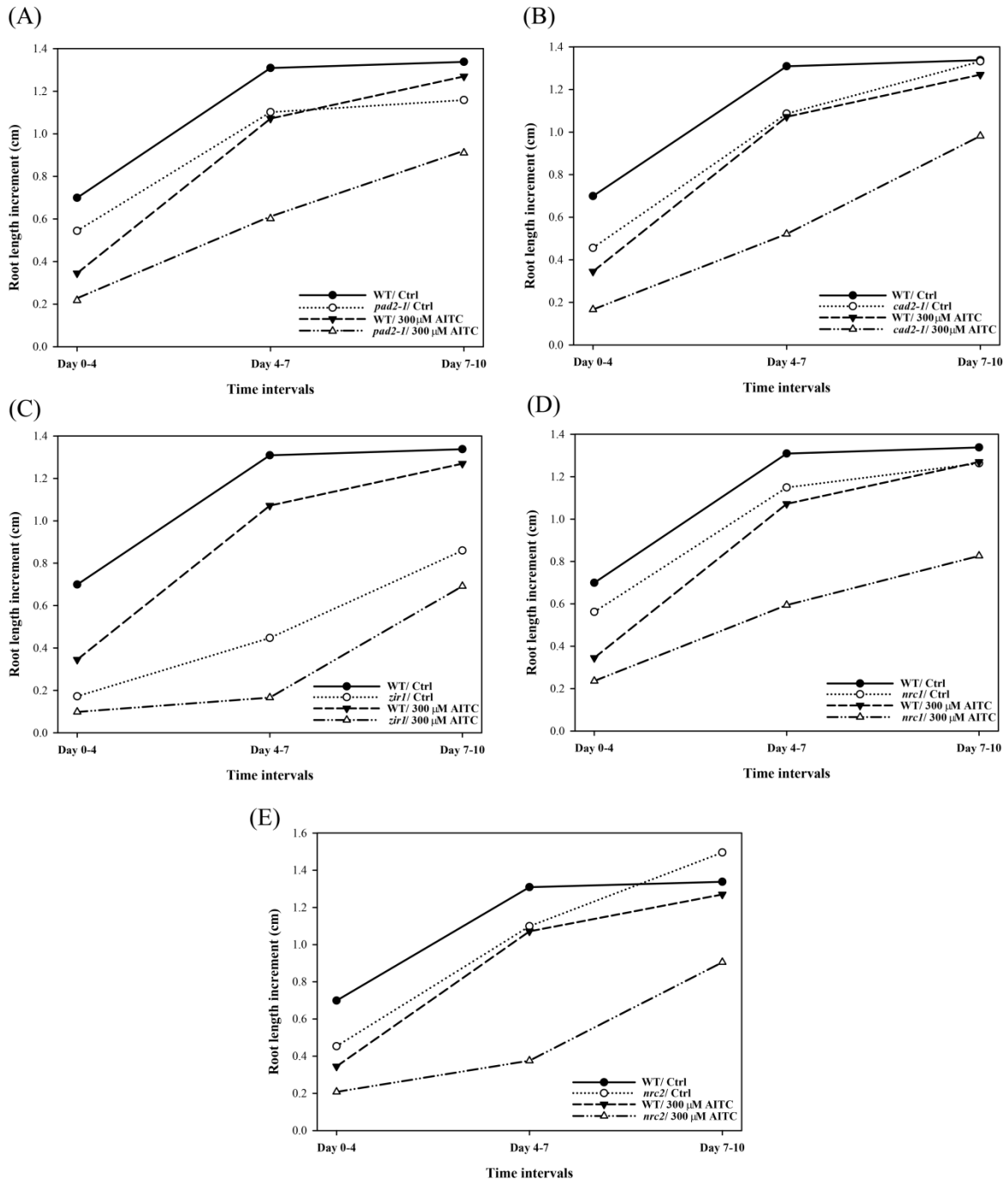
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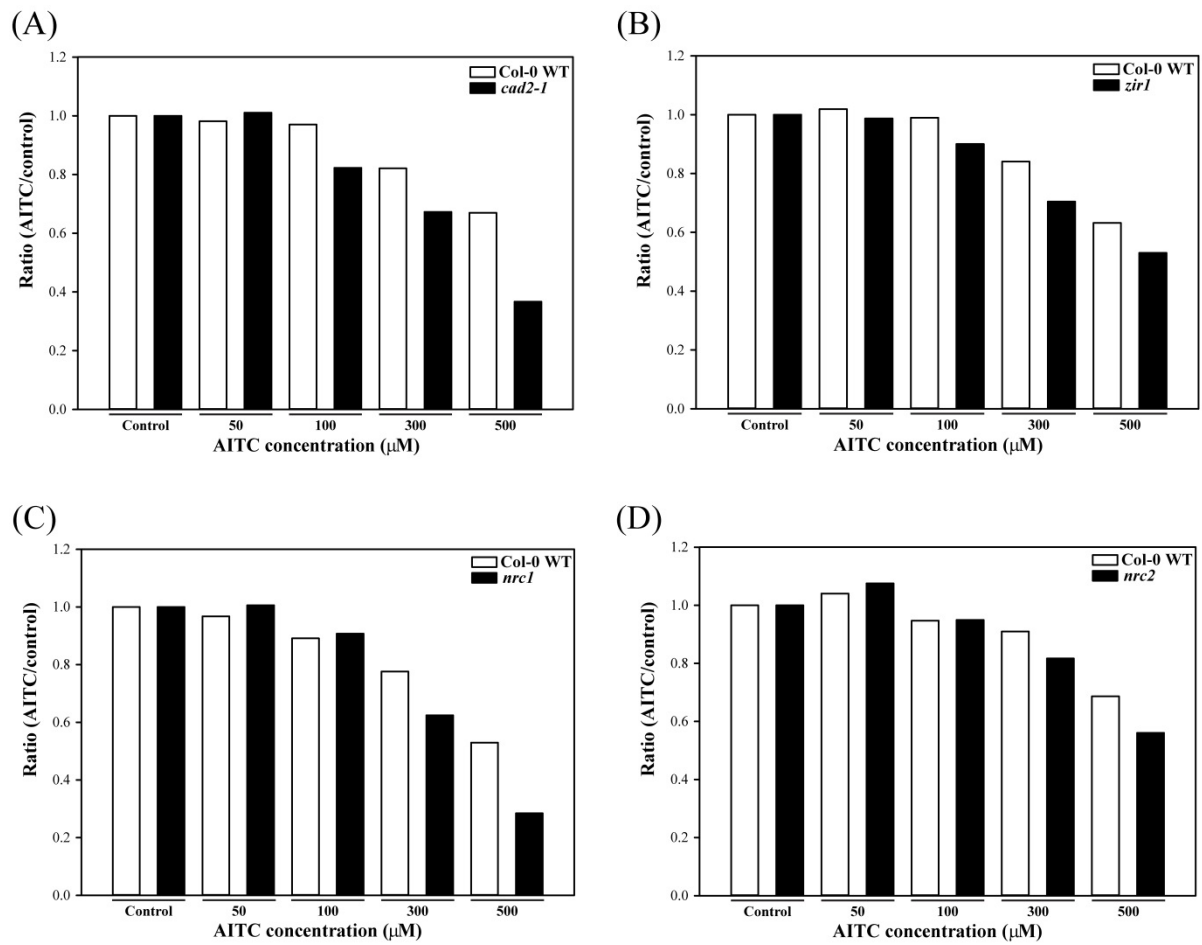
Supplementary Figure S1. The ratio between the mean root length under a given AITC concentration and that of the control treatment calculated for 10 day old seedlings of the Col-0 WT and the glutathione mutants (A) *cad2-1*, (B) *zir1*, (C) *nrc1*, (D) *nrc2*.



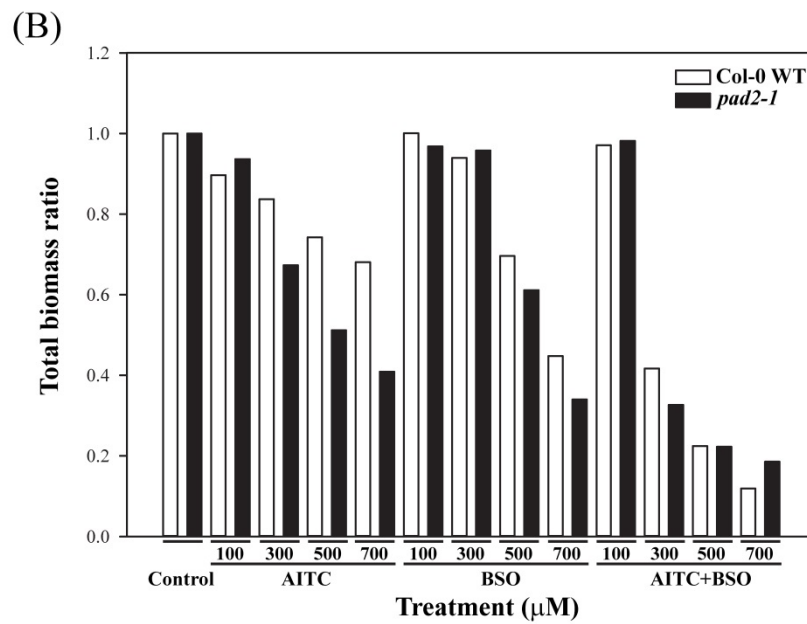
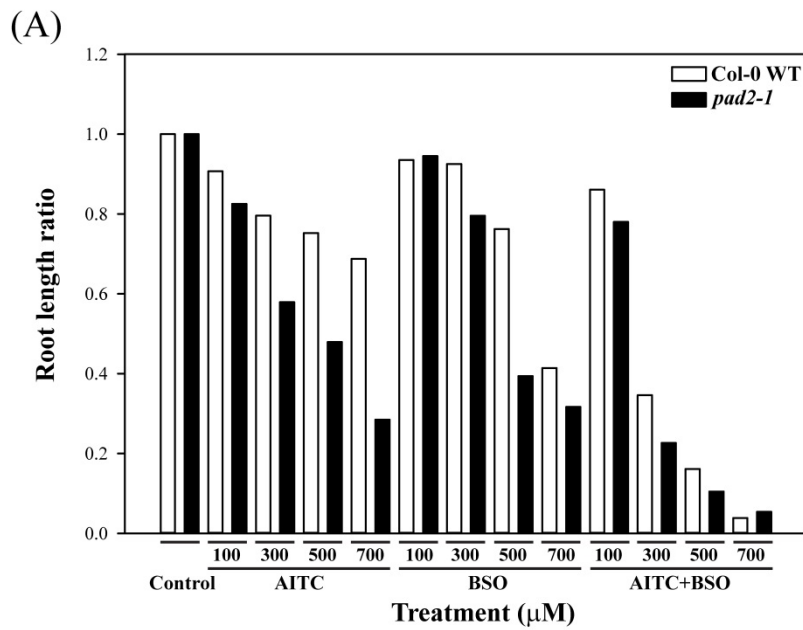
Supplementary Figure S2. Root elongation analysis over time using WT and glutathione mutants grown either on control medium or on medium supplemented with 300 μ M AITC: (A) *pad2-1*, (B) *cad2-1*, (C) *zir1*, (D) *nrc1* and (E) *nrc2*. Root lengths were measured (n= 48 for mutants; n= 96 for WT) at each 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*.

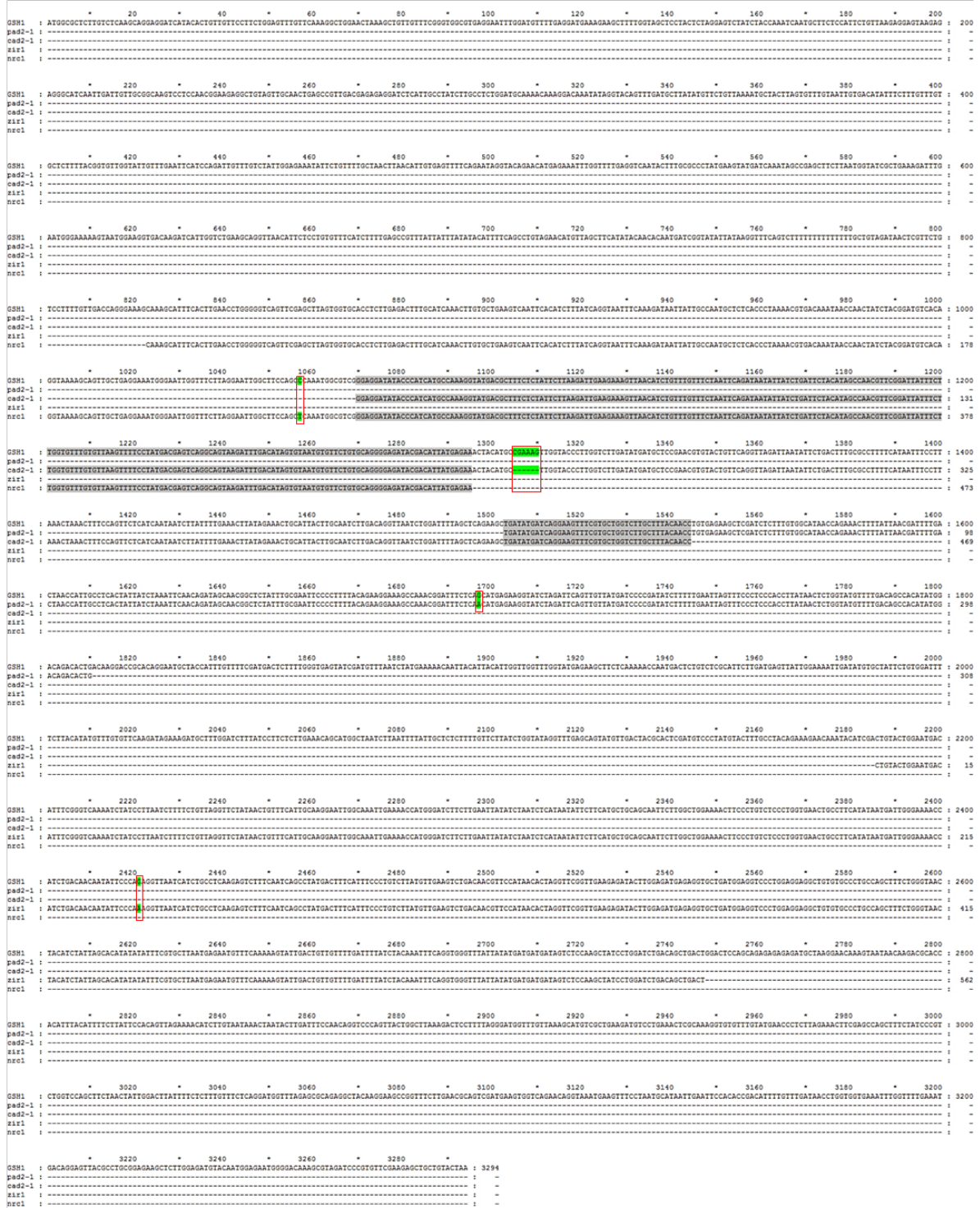


Supplementary Figure S4. Ratios of (A) mean root length and (B) mean biomass between a given treatment (AITC, BSO or AITC+BSO) and that of the control treatment calculated for 10 day old seedlings of the Col-0 WT and the glutathione mutant *pad2-1*.

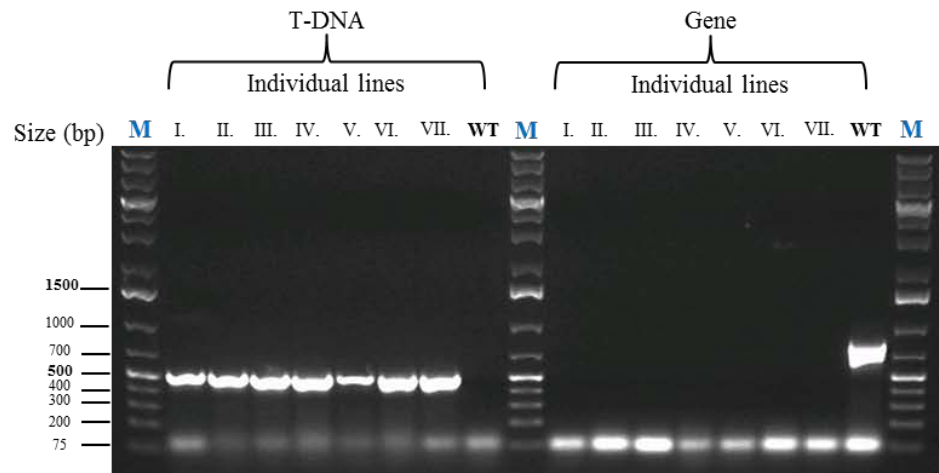


Supplementary Figure S5. Genotyping results of mutants used in this study (see also Supplementary Table S1) performed as described in more detail in the Methods section using the primers listed in Supplementary Table S2.

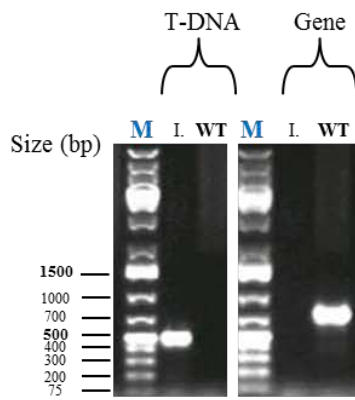
(A) *GSH1* deficient mutants *pad2-1*, *cad2-1*, *zir1* and *nrc1*



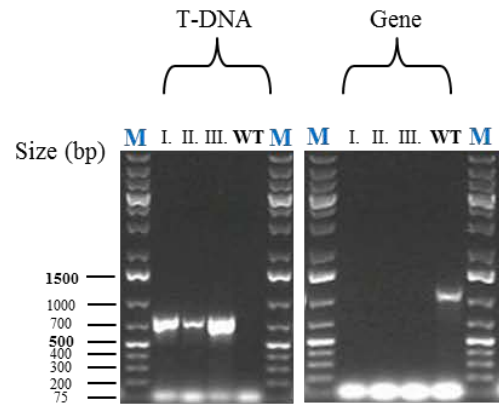
(C) *ggp1-1* (GK-319F10)



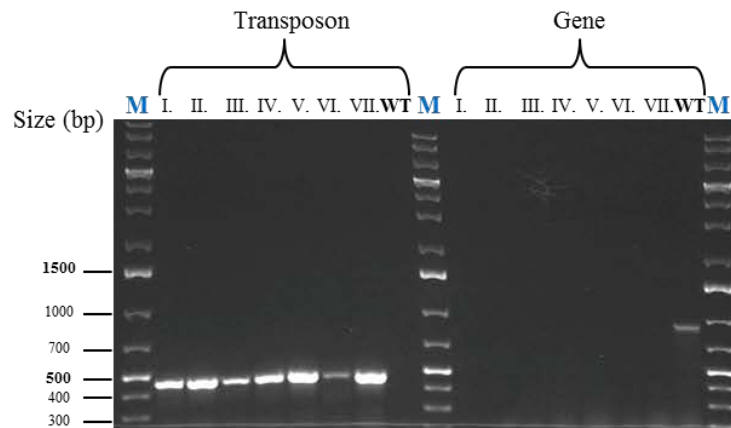
(D) *ggt1-1* (SAIL_1178_C01)



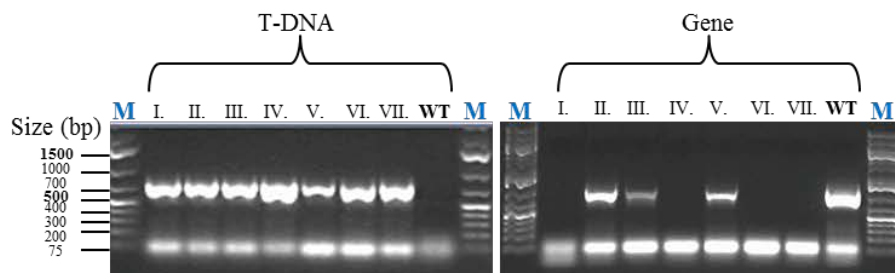
(E) *ggt2-1* (SAIL_6_G02)



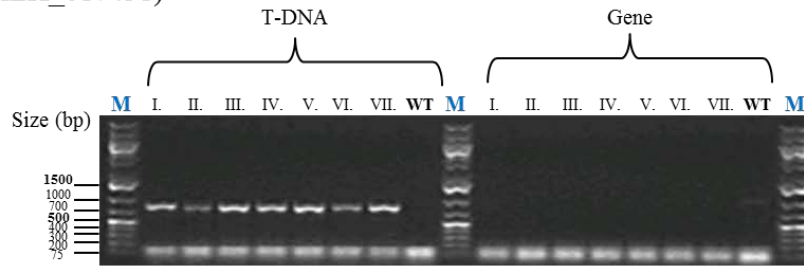
(F) *ggt3* (ET1113)



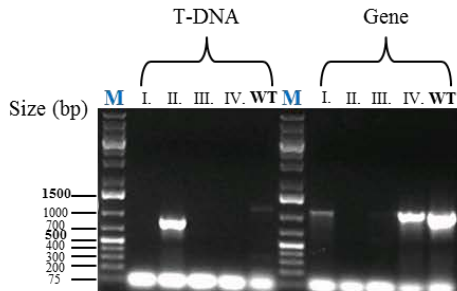
(G) *ggt4* (GT_5_57895)



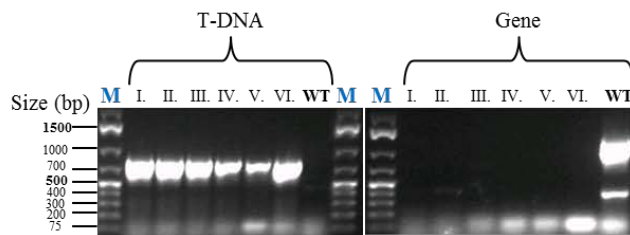
(H) *mrp1* (SALK_017431)



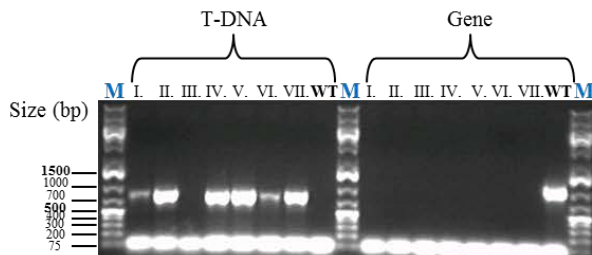
(I) *mrp2-2* (SALK_127425)



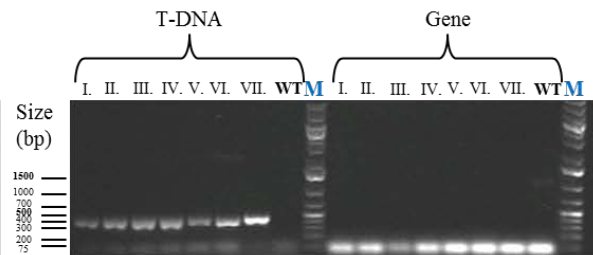
(J) *mrp3* (SALK_044022)



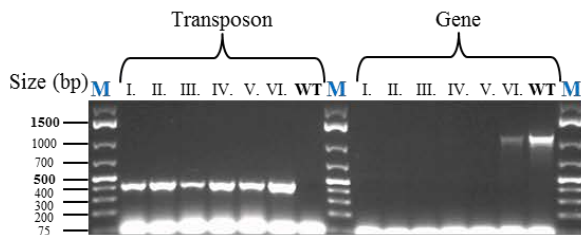
(K) *opt1-3* (SALK_052985)



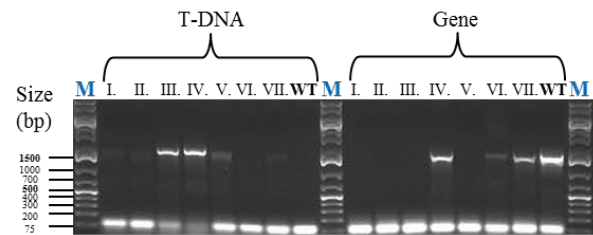
(L) *opt6* (SALK_201534)



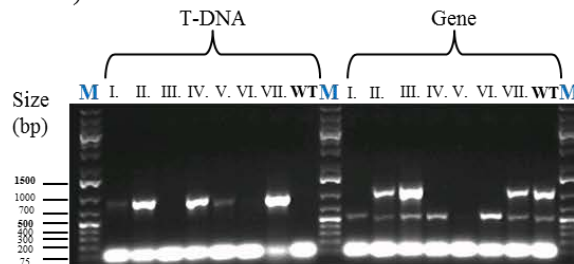
(M) *sultr1;2* (SM_3.30250)



(N) *sultr4;1* (SALK_103873)



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

<i>opt1-3</i>	SALK_052985	N662479	<i>OPT1</i>	AT5G55930	<i>oligopeptide transporter 1</i>	12
<i>opt6</i>	SALK_201534	N689065	<i>OPT6</i>	AT4G27730	<i>oligopeptide transporter 6</i> ; involved in the transport of glutathione derivatives	13
<u>pad2-1</u>		N3804	<i>GSH1</i>	AT4G23100	<i>phytoalexin deficient 2</i> ; γ -ECS/GSH1 is the first and rate-limiting enzyme of glutathione biosynthesis	14
<i>pcs1pcs2</i>		Grill	<i>PCS1</i> <i>PCS2</i>	AT5G44070 AT1G03980	<i>phytochelatin synthase 1 and 2</i> ; synthesis of phytochelatins and catabolism of glutathione conjugates	15
<i>sultr1;2</i>	SM_3.30250	N116961	<i>SULTR1;2/SEL1</i>	AT1G78000	<i>sulfate transporter 1;2</i> ; high-affinity sulfate transporter	16
<i>sultr4;1</i>	SALK_103873	N673083	<i>SULTR4;1</i>	AT5G13550	<i>sulfate transporter 4;1</i> ; low-affinity sulfate transporter	17
<i>sultr4;2</i>	SALK_103827	N676417	<i>SULTR4;2</i>	AT3G12520	<i>sulfate transporter 4;2</i> ; low-affinity sulfate transporter	18
<u>zir1</u>		Yeh	<i>GSH1</i>	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')
<i>cad2-1</i>	For GGAGGATATACCCATCATGC
	Rev GGTGTAAAGCAAGACCAGC
<i>ggp1-1</i>	For AGAATGATGATTGGATCCTTAAGC
	Rev GATCGCTATGCTATCAGGAATCTC
	T-DNA ATAATAACGCTGCGGACATCTACATTTT
<i>ggt1-1</i>	For GACTTGGCCTCAACGTATACATC
	Rev CGCGAGACTTAACAATACTTTGC
	T-DNA TAGCATCTGAATTTTCATAACCAATCTCGATACAC
<i>ggt2-1</i>	For CTCCCACCATTGTACTCAAGG
	Rev CTAGAGGCGGAGTATCCAAGG
	T-DNA TAGCATCTGAATTTTCATAACCAATCTCGATACAC
<i>ggt3</i>	For GTATCACTTCTTGGTCACACTGC
	Rev GATCAAATAACTTACCTGCCACC
	T-DNA CCGTTTTGTATATCCCCTTCCGT
<i>ggt4</i>	For AATCGGTGGTGGTTCTTTCTTGATT
	Rev TGTTGAGAAATCATCCATCTCGTTG
	T-DNA ACCCGACCGGATCGTATCGGT
<i>mrp1</i>	For ATTGGGGTTTTATGTGAAGC
	Rev ACTTCACTGTATCCATTGCCG
	T-DNA TGGTTCACGTAGTGGGCCATCG
<i>mrp2-2</i>	For GCTTTTCTGCGTTTGTAGTGG
	Rev TTTTCCAAGCATAACACCTG
	T-DNA TGGTTCACGTAGTGGGCCATCG
<i>mrp3</i>	For AAGTCAGCAATTGCTTTGGAG
	Rev AGGGAGCCTTTTATGCTCAC
	T-DNA TGGTTCACGTAGTGGGCCATCG
<i>nrc1</i>	For CAAAGCATTTCACTTGAACC
	Rev TTCTCATAATGTCGTATCTCCC
<i>nrc2</i>	For TGCTAGGCTGCTTATTGAGG
	Rev TCCCTCACATCATCTCCATAG
<i>opt1-3</i>	For AAAACCAAAGAAAACGGATCC
	Rev GACTATTACCCACAACAACAAAATG
	T-DNA TGGTTCACGTAGTGGGCCATCG
<i>opt6</i>	For AAAATTCAAACATTACAAGCCATG
	Rev GATAGGGAAGGTCTTGGCATC
	T-DNA TGGTTCACGTAGTGGGCCATCG
<i>pad2-1</i>	For TGATATGATCAGGAAGTTTCG
	Rev CAGTGTCTGTCCATATGTGG
<i>pcs1pcs2</i>	For _{pcs1} CGAATCCACTAACGAATCTTCC
	Rev _{pcs1} TTGATTCATCAAACCACCTCC
	For _{pcs2} GGTTACGAATTATTGATAGCC
	Rev _{pcs2} CTCTGTTCTTCGAATCTCAGC
	T-DNA ATGGCGAAATCAAGGCATC
<i>sultr1;2</i>	For ATTGGTGCATCATTCTTGACC
	Rev TCCGAGAACTGCAGTTCTAGG
	T-DNA CTTATTTTCAGTAAGAGTGTGGGGTTTTGG
<i>sultr4;1</i>	For AGCTAAGACTGGCCTATCAGG
	Rev CGTTTTGGTGAATCTTGCTC
	T-DNA TGGTTCACGTAGTGGGCCATCG
<i>sultr4;2</i>	For CCAGCAGCATGTATCGGTATC
	Rev ACCGTATATTGGTTGAAGCCC
	T-DNA TGGTTCACGTAGTGGGCCATCG
<i>zir1</i>	For CTGTACTGGAATGACATTTCCGG
	Rev AGTCAGCTGTCAGATCCAGG