			A. thaliana Gene ID	B. rapa				
	Gene	Encoded enzyme		GenBank acc. no.	Gene ID	Forward primer (5' to 3')	Reverse primer (5' to 3')	References
Internal control	PP2A			EX054539	Bra025162	AGGTGACACTATAGAATAATTTGATGCAATATTCTGAACTCTCA	GTACGACTCACTATAGGGAACAACTTAGGCTGTTTGCATGA	Chen et al. (2010) Anal Biochem 405:138-140
	TIP41			EX117271	Bra011516	AGGTGACACTATAGAATAACCTGAGGGGGAAGCTAGTC	GTACGACTCACTATAGGGACTCCATTGTCAGCCAGTTCA	Chen et al. (2010) Anal Biochem 405:138-140
	Actin			EX139908	Bra037560	AGGTGACACTATAGAATAACATTCCAGCAGATGTGGATCTC	GTACGACTCACTATAGGGAAACCCAGAGAGTTTTGTCACAC	Schuller and Ludwig-Müller (2006) New Phytologist 171: 145-15
AsA synthesis	MIOX	Myoinositol oxygenase	At4g26260	EX132809	Bra041014	AGGTGACACTATAGAATAGCTGGCTATGAAGTAATTAGTCCTG	GTACGACTCACTATAGGGAACTCGCTATGGATGATTGTGA	
	PMI	Phosphomannose isomerase	At1g42550	ES934679	Bra021430	AGGTGACACTATAGAATAGGGAACTCGCAATTGAGTC	GTACGACTCACTATAGGGACACTGGAGCCATCAGCAT	
	GMP	GDP-D-mannose pyrophosphorylase	At2g39770	EX141191	Bra005014	AGGTGACACTATAGAATATTTTCATCTTTGTGCTTTCGG	GTACGACTCACTATAGGGATTACAAAACACCAAATGACTTTTAAC	
	GGP	GDP-L-galactose phosphorylase	At4g26850	EX092676	Bra010424	AGGTGACACTATAGAATATTCTCCGGCGAAAATTGG	GTACGACTCACTATAGGGATGCGGCAAAGGGTTAGTCT	
	GPP	L-galactose-1-phosphate phosphatase	At3g02870	EX061908	Bra040607	AGGTGACACTATAGAATAAAGCTGGACAGGTGATTCGT	GTACGACTCACTATAGGGAACCATTCGCAGCTGTTGTTT	
	GalDH	L-galactose dehydrogenase	At4g33670	EX133611	Bra036989	AGGTGACACTATAGAATATGGGAAACACAGGTCTCAAA	GTACGACTCACTATAGGGATTAATGCCTTGTCGGAACG	
	GLDH	L-galactono-1,4-lactone dehydrogenase	At3g47930	ES933110	Bra018100	AGGTGACACTATAGAATAATACGCAGCACTGGCTCTCT	GTACGACTCACTATAGGGAGTCTCCGGCTGGTTAAAGTTC	
	GalUR	D-galacturonate reductase		EX083199	Bra027828	AGGTGACACTATAGAATATGAAGTGGAGGAATGCAAGA	GTACGACTCACTATAGGGAGCAGTCAAAACAATGCCCTT	Agius et al. (2003) Nat Biotechnol 21:177-181
AsA oxidation	APX3	Ascorbate peroxidase 3	At4g35000	EX121179	Bra013053	AGGTGACACTATAGAATACAACGTCTACAGTCACTATGGC	GTACGACTCACTATAGGGAGAGAAGAGAAGAGGGGAATGCGT	
	APX4	Ascorbate peroxidase 4	At4g09010	DY010066	Bra000663	AGGTGACACTATAGAATACTGCATTGACAAAGCTAAGTGG	GTACGACTCACTATAGGGATTGTTGTCACGCTTATGCTCA	
	tAPX	Thylakoidal ascorbate peroxidase	At1g77490	EX054285	Bra015668	AGGTGACACTATAGAATATTCCACCCAAAAGAAAGAAGAGC	GTACGACTCACTATAGGGAACAAGAGGACCAAAACGCTG	
	AO	Ascorbate oxidase	At5g21105	XM_009128252	Bra020145	AGGTGACACTATAGAATAGGGAAGAAAGAGCCATTGA	GTACGACTCACTATAGGGATGTTGGTAACGATGTGCTGC	
AsA recycling	MDHAR	Monodehydroascobate reductase	At3g27820	EX100231	Bra025303	AGGTGACACTATAGAATAGGTGAGTCAGCATATAGTTCCAG	GTACGACTCACTATAGGGAGAGGCTTCTCCATCATCACCA	
	DHAR1	Dehydroascobate reductase	At1g19570	EX046186	Bra008662	AGGTGACACTATAGAATAACTAGTTCCATCTGCTCCGC	GTACGACTCACTATAGGGAAGCATAATAGATTGTTTGGTTAGAACA	
	DHAR2	Dehydroascobate reductase	At1g75270	EE521888	Bra008188	AGGTGACACTATAGAATACGTGCGAAATCTGAAGATCG	GTACGACTCACTATAGGGACGGAGACGTTAGCGAGATGA	
	GR	Glutathione reductase		AF008441	Bra015006	AGGTGACACTATAGAATACACACACCATCTTAGAGAGTTTGG	GTACGACTCACTATAGGGATCATAGTGAGTCTCGGTTGTAGC	Lee et al. (1998) Biochim Biophys Acta 1395:309-314
Defense	PR1		At2g14610	DN237938	Bra013123	AGGTGACACTATAGAATAACGTGCAATGGAGAATGCCT	GTACGACTCACTATAGGGATTGCCCCGAGGATCATAGTT	
	PDF1.2b		At2g26020	EX083692	Bra015809	AGGTGACACTATAGAATATCTACCAAAATCATGGGTGCT	GTACGACTCACTATAGGGACTGCTACATGTCATGATGTCACTC	
	RDR1		At1g14790	EX127661	Bra026187	AGGTGACACTATAGAATAAATCTATTGGAAGGGCGGTT	GTACGACTCACTATAGGGATCACATGATACCAAGCCGAG	
	RDR6		At3g49500	EV015278	Bra029957	AGGTGACACTATAGAATACCCTGAAGAAAGAGCTGAGG	GTACGACTCACTATAGGGAAAGCAAAGCTCAGCATCACC	
JA responsive	AF528182	Glucosyltransferase-like protein		AF528182		AGGTGACACTATAGAATACCTGACGTCATCGAGCTTCT	GTACGACTCACTATAGGGAATCACACAAGCAGCAGTTGG	Park et al. (2003) Plant Cell Rep 21: 1027-1034
	AF528183	Putative metal-binding farnesylated protein	1	AF528183		AGGTGACACTATAGAATAGCAGAGCACTGGGAAAAAGA	GTACGACTCACTATAGGGATCCGACTTCCTCACAAAACC	Park et al. (2003) Plant Cell Rep 21: 1027-1034



Proposed pathways for ascorbic acid biosynthesis, oxidation and recycling in plants. Arrows indicate a series of enzymatic reactions on each pathway, and the genes encoding each enzyme studied in this study are detailed on the pathways.

PMI, phosphomannose isomerase

GMP, GDP-D-mannose pyrophosphorylase

GGP, GDP-L-galactose phosphorylase

GPP, L-galactose-1-phosphate phosphatase

GalDH, L-galactose dehydrogenase

GLDH, L-galactono-1,4-lactone dehydrogenase

MIOX, myoinositol oxygenase

GalUR, D-galacturonate reductase

APX, ascorbate peroxidase

AO, ascorbate oxidase

MDHAR, monodehydroascorbate reductase

DHAR, dehydroascorbate reductase

GR, glutathione reductase



Expression level of defense genes in turnip (*Brassica rapa* subsp. *rapa*) cv. CR Mochibana after treatment with 10 mM L-galactose. Transcript levels were determined with quantitative RT-PCR 24 h after treatment. Error bars indicate standard error for biological triplicates. PR, pathogenesis-related protein; PDF, plant defensin; RDR, RNA-dependent RNA polymerase.



Viral accumulation of *Turnip mosaic virus* strain TuR1-YFP in noninoculated upper leaves of an *Arabidopsis thaliana* ecotype Columbia (Col-0) and the *ao* mutant. Error bars indicate standard error for three to four biological replicates. Asterisks represent significant differences determined by Student's *t*-test (* $P \le 0.05$).

Symptoms on inoculated and uninoculated upper leaves of the five Chinese cabbage (*Brassica rapa* subsp. *pekinensis*) and turnip (*Brassica rapa* subsp. *rapa*) cultivars with different alleles at the *Rnt1* locus. Second true leaves of each plant were inoculated with *Turnip mosaic virus* (TuMV) strain UK1. Chinese cabbage cvs. Aki-masari and Ku-kai 65 with resistance gene *Rnt1-1* did not develop lesions. In Chinese cabbage cv. Yu-shun carrying *rnt1-2*, necrotic lesions had developed by 5 days post inoculation (DPI) and expanded more by 8 DPI. At 14 DPI, the plants were systemically infected. Turnip cv. CR Mochibana carrying *rnt1-3* did not have any visible symptoms at 4 DPI, but chlorotic and necrotic spots had appeared on inoculated leaves by 8 DPI; by 14 DPI, mosaic symptoms were observed on uninoculated upper leaves. Turnip cv. Yukihime-kabu carrying *rnt1-3* had symptoms similar to those on CR mochibana, but yellowing of inoculated leaves developed earlier.



Aki-masari (*Rnt1-1/rnt1-3*)





Uninoculated upper leaves



TuMV-UK1



Mock

TuMV-UK1

Yu-shun (*rnt1-2*/*rnt1-2*)





Mock TuMV-UK1

Uninoculated upper leaves



TuMV-UK1

CR Mochibana (*rnt1-3*/*rnt1-3*)





Mock

Uninoculated upper leaves



TuMV-UK1

Yukihime-kabu (*rnt1-3*/*rnt1-3*)

TuMV-UK1



Mean TAA (ascorbic acid [AS] and dehydroascorbic acid [DHA]) content in the five Chinese cabbage (*Brassica rapa* subsp. *pekinensis*) and turnip (*Brassica rapa* subsp. *rapa*) cultivars carrying different alleles at the *Rnt1* locus after inoculation with *Turnip mosaic virus* strains UK1 or TuR1-YFP. TAA content in the inoculated second true leaves was quantified 3 or 4 days after inoculation. The genotype at the *Rnt1* locus in each cultivar is described in parentheses. Hatched boxes, AS; white boxes, DHA. Error bars indicate standard error for biological triplicates for TAA content. An asterisk represents a significant difference determined by Student's *t*-test (* $P \le 0.05$). FW, fresh mass.



Mean TAA (ascorbic acid [AS] and dehydroascorbic acid [DHA]) content in the *Arabidopsis thaliana* ecotype Col-0 and the *ao* mutant after inoculation with *Turnip mosaic virus* strain TuR1-YFP. TAA content in non-inoculated upper leaves was quantified 10 days after inoculation. Error bars indicate standard error for biological triplicates for TAA content. FW, fresh mass.

Expression profiles determined with quantitative RT-PCR for genes for ascorbic acid (AS) synthesis, oxidation and recycling in turnip (*Brassica rapa* subsp. *rapa*) cvr. (A) Yukihime-kabu infected with *Turnip mosaic virus* (TuMV) strain UK1 at 4 days post inoculation (DPI) and (B) CR Mochibana infected with TuMV strain TuR1-YFP at 3 DPI. Asterisks represent significant differences determined by Student's t-test (* $P \le 0.05$; ** $P \le 0.01$). See Table S1 and Fig. S1 for full name of each gene and locations within the AS synthesis, oxidation and recycling pathways.







Mean TAA [ascorbic acid (AS) and dehydroascorbic acid (DHA)] content in second true leaves of Chinese cabbage (*Brassica rapa* subsp. *pekinensis*) cv. Aki-masari at 24 h post treatment (HPT) with H₂O₂, salicylic acid (SA), methyl-jasmonate (MeJA) or abscisic acid (ABA) treatments. In the H₂O₂ treatment, TAA levels were also quantified at 1, 6 and 12 HPT. Hatched boxes, AS; white boxes, DHA. Error bars indicate standard error for biological triplicates for TAA content. An asterisk represents a significant difference determined by the Student's *t*-test (* $P \le 0.05$); FW, fresh mass.





Expression change of *PDF1.2* in Chinese cabbage (*Brassica rapa* subsp. *pekinensis*) cv. Akimasari after treatment with 50 μ M methyl-jasmonate (MeJA). Transcript levels were determined by quantitative RT-PCR 24 h after treatment. Error bars indicate standard error for biological triplicates. An asterisk represents a significant difference determined by Student's t-test (**P \leq 0.01).



Expression levels of the AF528182 and AF528183 genes in turnip (*Brassica rapa* subsp. *rapa*) cv. CR Mochibana by infection of *Turnip mosaic virus* (TuMV) strain TuR1-YFP. Transcript levels were determined by quantitative RT-PCR 3 days after inoculation. Error bars indicate standard error for biological triplicates.



Expression levels of the VTC1 and VTC2 genes in Chinese cabbage (*Brassica rapa* subsp. *pekinensis*) cv. Aki-masari after treatment with 50 μ M methyl-jasmonate (MeJA). Transcript levels were determined by quantitative RT-PCR 24 h after treatment. Error bars indicate standard error for biological triplicates.