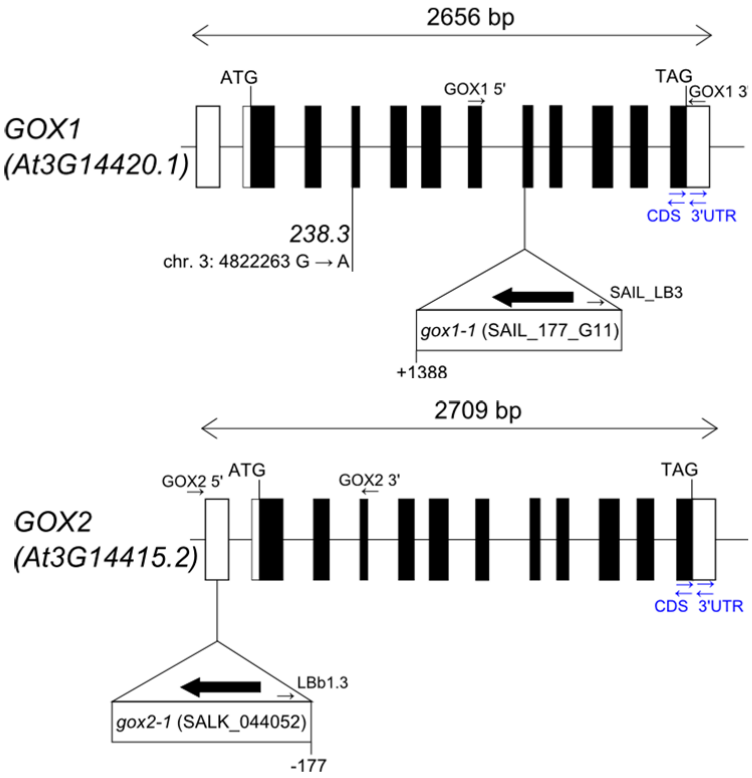
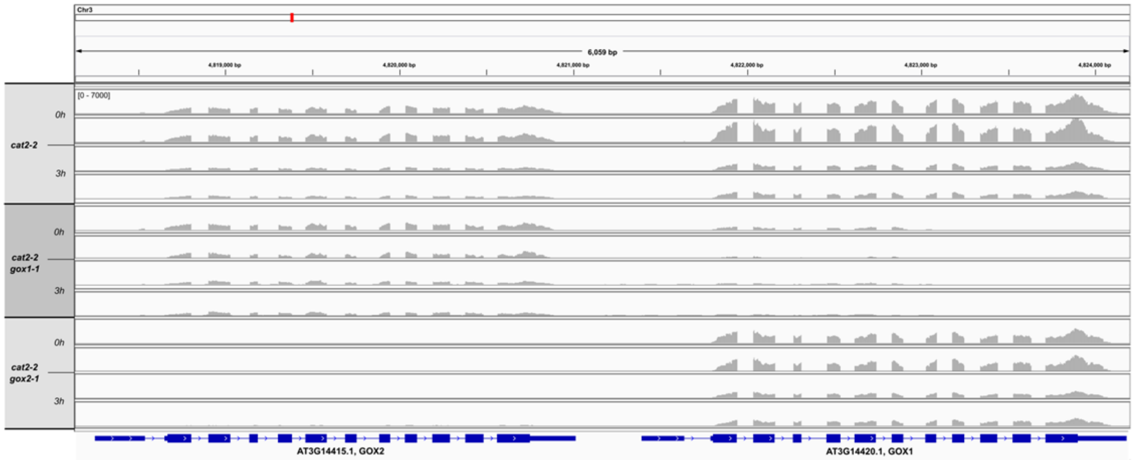
Supplemental Figure S1.



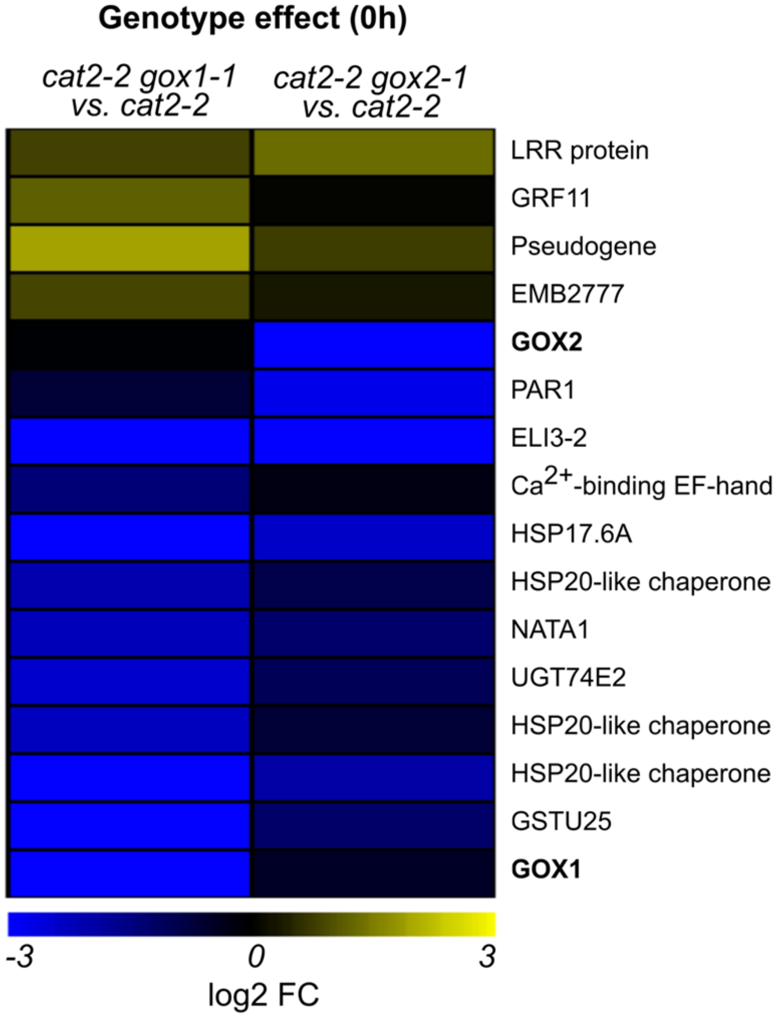
Supplemental Figure S1. *GOX1* and *GOX2* gene models, together with T-DNA insertion sites in the knockout SAIL_177_G11 (*gox1-1*) and SALK_044052 (*gox2-1*) lines, as well as the EMS-induced single nucleotide polymorphism in line *238.3*. Black and white boxes represent protein-coding and untranslated regions, respectively. Black arrows indicate primers used for genotyping and blue arrows – primers used for qRT-PCR.

Supplemental Figure S2.



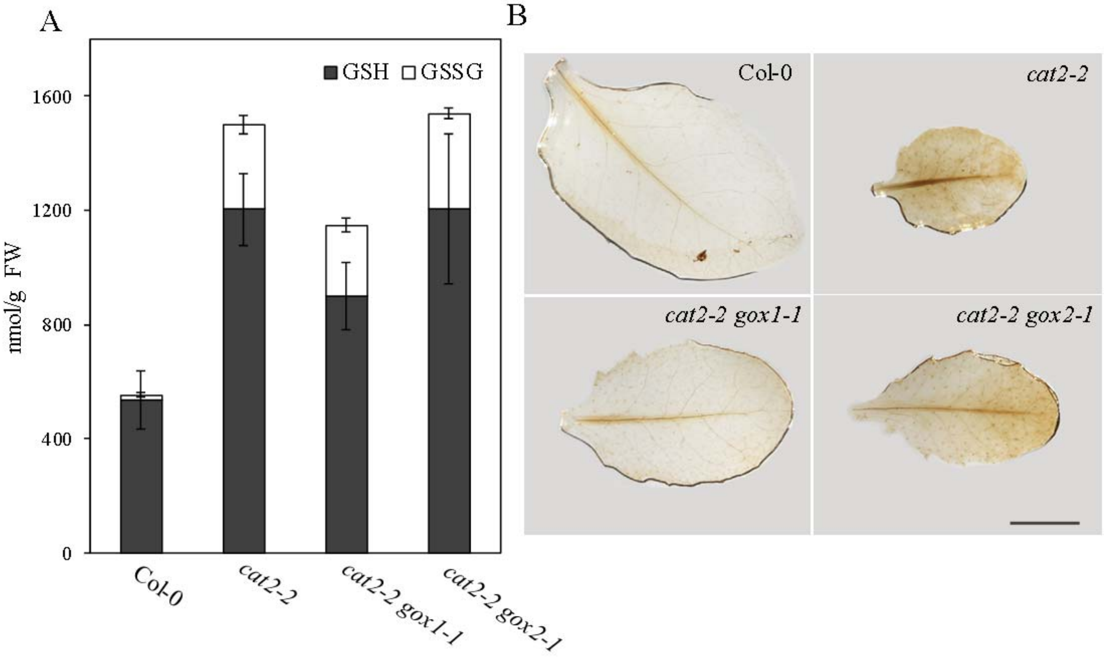
Supplemental Figure S2. Read coverage of *GOX1* and *GOX2* loci from RNA-seq analysis of *cat2-2 gox1-1*, *cat2-2* and *cat2-2 gox2-1* mutants. Aligning of reads obtained by RNA-seq from *cat2-2*, *cat2-2 gox1-1* and *cat2-2 gox2-2* mutants before (0h) and after exposure to photorespiratory stress (3h) to the reference sequences of *GOX1* and *GOX2*.

Supplemental Figure S3.



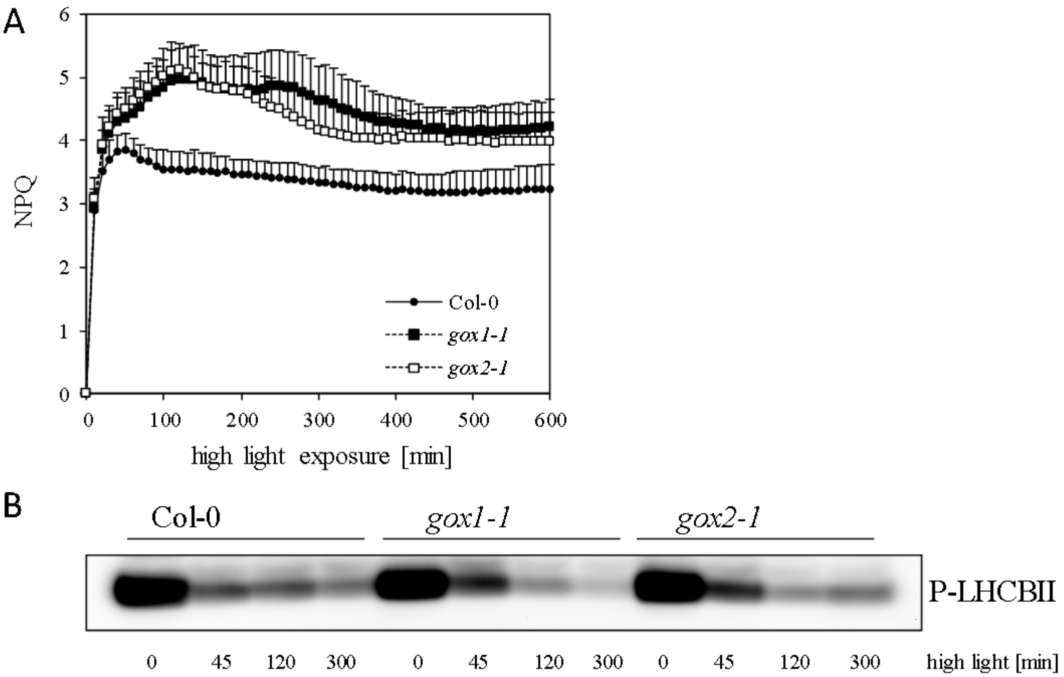
Supplemental Figure S3. Heat map of differentially expressed transcripts (|log2 FC| > 1, FDR < 0.05) between *cat2-2 gox1-1*, *cat2-2 gox2-1* and *cat2-2* mutants under control high CO_2 conditions aimed at inhibiting the photorespiratory flux.

Supplemental Figure S4.



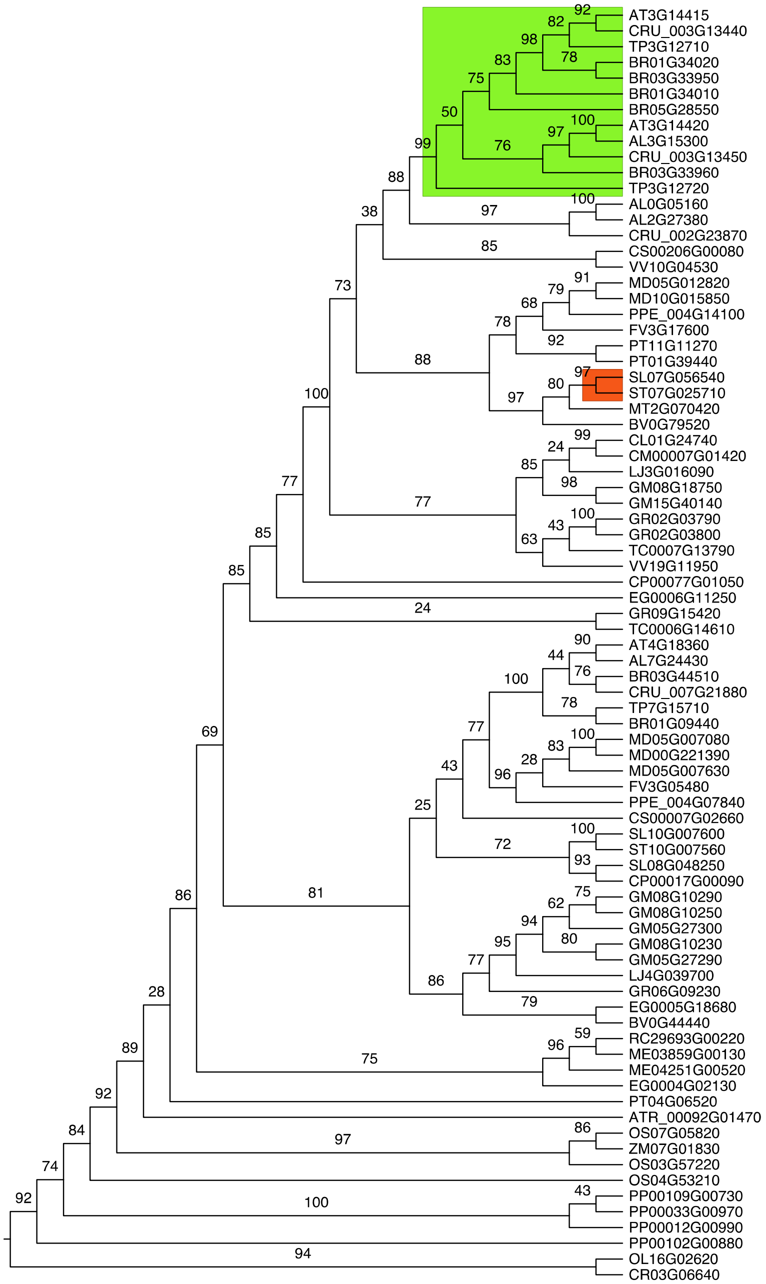
Supplemental Figure S4. Redox status of Col-0, *cat2-2*, *cat2-2 gox1-1* and *cat2-2 gox2-1* plants grown under ambient air and moderate light intensity (300 µmol m^{-2} s^{-1}). A) Levels of reduced and oxidized glutathione (GSH and GSSG, respectively) in rosettes of three-week-old plants. Bars represent means of three biological replicates ± SE. (B) Representative bright-field images of 3,3′-diaminobenzidine-stained mature leaves of three-week-old plants. The scale bar is 0,5 cm.

Supplemental Figure S5.



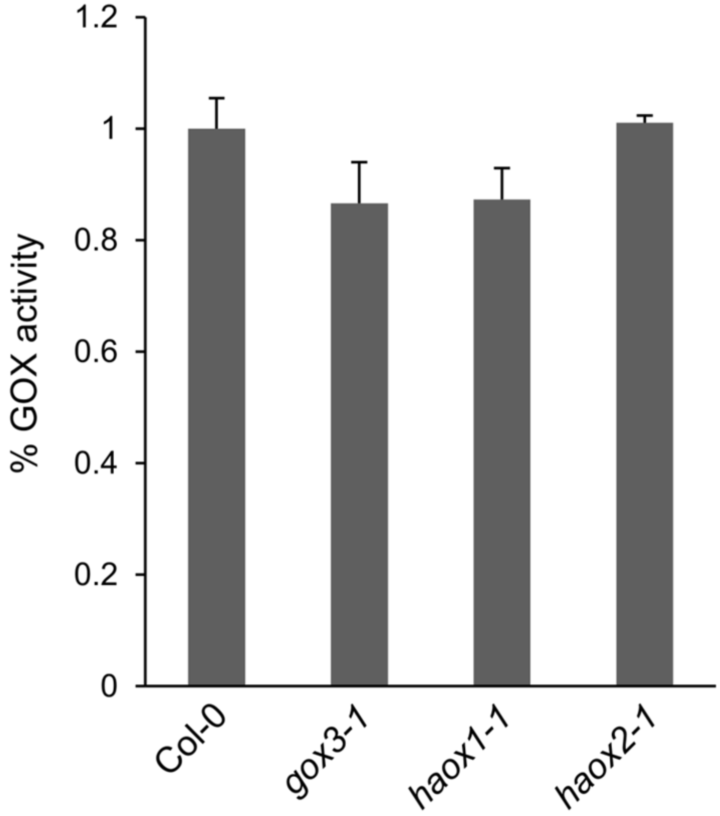
Supplemental Figure S5. Non-photochemical quenching (NPQ; A) and phosphorylation of LHCBII (B) upon exposure of Col-0, *gox1-1* and *gox2-1* plants grown under conditions limiting photorespiration to high light.

Supplemental Figure S6.



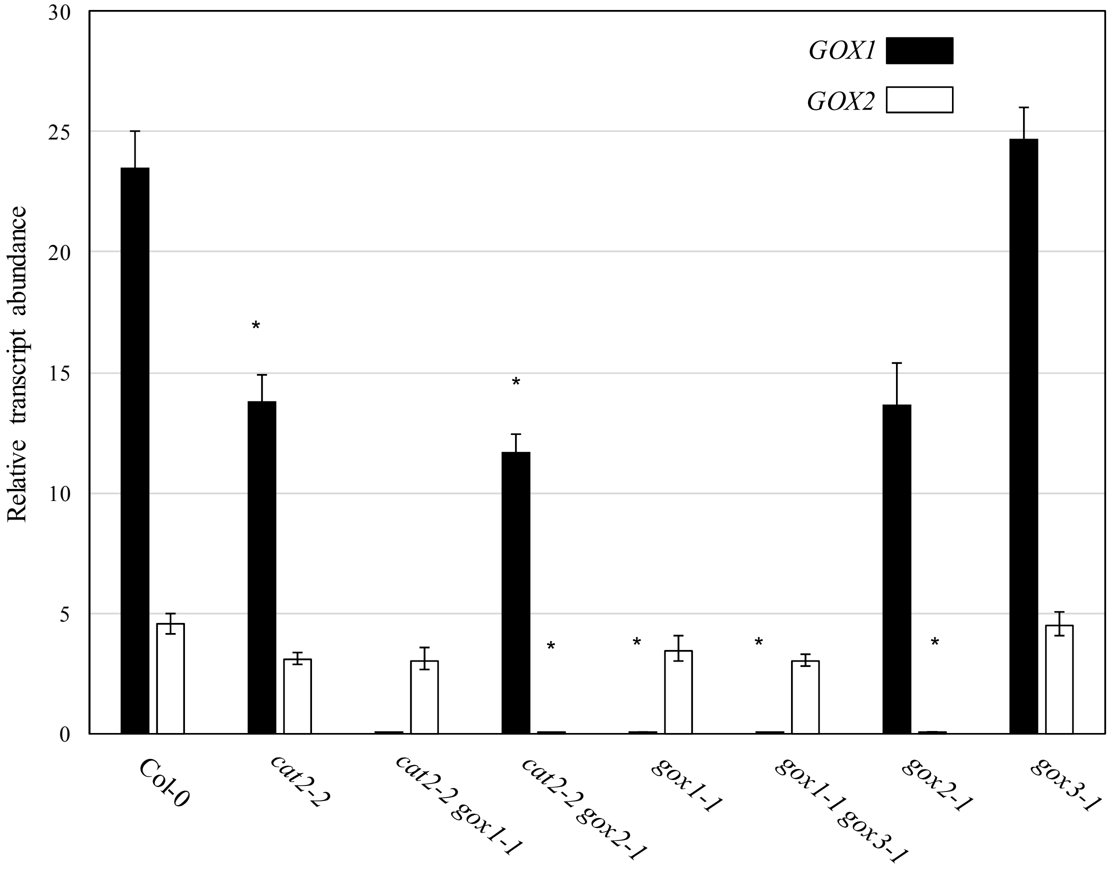
Supplemental Figure S6. *GOX1* and *GOX2* genes in *Brassicaceae* are clustered as monophyletic group in the gene tree of ORTHO03D000507 from PLAZA 3.0 Dicots. *GOX1* and *GOX2* in *Brassicaceae* are in green, and the two *GOX* genes from *Solanaceae* identified based on synteny are shown in red. Numbers on branches denote bootstrap values.

Supplemental Figure S7.



Supplemental Figure S7. Extractable leaf GOX activity. Bars represent averages from three biological replicates ± SE.

Supplemental Figure S8.



Supplemental Figure S8. *GOX1* and *GOX2* transcript abundance. RNA was extracted from rosettes of three-week-old plants grown *in vitro* and used to quantify gene expression levels by qRT-PCR. Values are means ± SD of four biological replicates. Asterisks indicate significant difference ($p<0.05$) in comparison to Col-0 according to one-way ANOVA.

Supplemental Table S1. Maximum likelihood estimates of parameters under branch models on both post-duplication branches leading to the *GOX1* and *GOX2* clades.

Model	p	ℓ	κ	ω_0	ω_{GOX1}	ω_{GOX2}
A: One ratio: $\omega_0 = \omega_{GOX1} = \omega_{GOX2}$	33	-5437.64	1.71	0.070	0.070	0.070
B: Two ratios: $\omega_0 = \omega_{GOX1}$, ω_{GOX2}	34	-5432.37	1.69	0.067	0.067	31.293
C: Two ratios: $\omega_0 = \omega_{GOX2}$, ω_{GOX1}	34	-5436.67	1.71	0.069	0.233	0.069
D: Two ratios: ω_0, $\omega_{GOX1} = \omega_{GOX2}$	34	-5433.50	1.71	0.067	0.291	0.291
E: Three ratios: ω_0, ω_{GOX1}, ω_{GOX2}	35	-5432.21	1.70	0.066	0.105	3.941
F: Two ratios: $\omega_0 = \omega_{GOX1}$, $\omega_{GOX2} = 1$	33	-5432.55	1.70	0.067	0.067	1.000
G: Two ratios: $\omega_0 = \omega_{GOX2}$, $\omega_{GOX1} = 1$	33	-5437.34	1.72	0.068	1.000	0.068
H: Two ratios: ω_0, $\omega_{GOX1} = \omega_{GOX2} = 1$	33	-5436.17	1.72	0.066	1.000	1.000
I: Three ratios: ω_0, ω_{GOX1}, $\omega_{GOX2} = 1$	34	-5432.30	1.70	0.067	0.116	1.000
J: Three ratios: ω_0, ω_{GOX2}, $\omega_{GOX1} = 1$	34	-5435.07	1.73	0.066	1.000	0.275

p, number of free parameters in the model; ℓ, log-likelihood; κ, the ratio of transition to transversion; ω_{GOX1} and ω_{GOX2}, the ratio of non-synonymous substituion rate (dN) to synonymous substituion rate (dS) on the two post-duplication branches to *GOX1* and *GOX2* clades, respectively; ω_0, dN/dS for rest of the branches on the tree

Supplemental Table S2. Likelihood ratio tests for comparing different branch models applied to both post-duplication branches leading to the *GOX1* and *GOX2* clades.

Model comparisons	**$2\Delta\ell$**	**df**	***p* value**
A-D	8.295754	1	0.003973788
A-B	10.542752	1	0.001166446
C-E	8.923886	1	0.00281465
A-C	1.944748	1	0.16315393
B-E	0.325882	1	0.568094036
D-H	5.342664	1	0.020809648
B-F	0.348118	1	0.555180423
E-I	0.177828	1	0.673246506
C-G	1.331848	1	0.248476723
E-J	5.729078	1	0.016686241

$2\Delta\ell$, likelyhood ratio statistics; df, degree of freedom

Supplemental Table S3. The modified branch-site models for detecting positive selection on six branches after the duplication event leading to *GOX2* in *Arabidopsis*.

Branch	ℓ_{H0}	ℓ_{HA}	$2\Delta\ell$	**df**	***p* value**
#1	-5401.887282	-5401.781842	0.21088	1	0.32303927
#2	-5401.767414	-5401.577101	0.38062	1	0.26863420
#3	-5402.937338	-5402.85316	0.16835	1	0.34078851
#4	-5403.195792	-5403.195793	2E-06	1	0.49943581
#5	-5403.170388	-5403.170388	0	1	0.5
#6	-5402.919624	-5402.047157	1.74493	1	0.09325743

ℓ_{H0}, log likelihood for the branch-site model without positive selection; ℓ_{HA}, log likelihood for the branch-site model with positive selection; $2\Delta\ell$, likelyhood ratio statistics; df, degree of freedom

Supplemental Table S4. List of primers used in the study.

Primer name	Use	Sequence
gox1_SAIL_177_G11_newLP1	Genotyping	GGGAATCACAAGAAATTGATC
gox1_SAIL_177_G11_RP	Genotyping	GAAGGACTTGACCTCGGAAAG
gox2_SALK_044052_LP	Genotyping	ATACCATGATGGTTCCAGCAG
gox2_SALK_044052_RP	Genotyping	TTGTTATGTTTCGTCAAGCCC
gox3_GABI_523D09_LP	Genotyping	TTGGATGGGAATACTTTATTGG
gox3_GABI_523D09_RP	Genotyping	GCATCAACCTTTTGCTTGAAG
haox1_SAIL_84_A04_newLP2	Genotyping	AGAGGACAAGAGGAGCAAGATTC
haox1_SAIL_84_A04_RP	Genotyping	GCGCTTTGAAAACATCTGTTC
haox2_SALK_102409_LP	Genotyping	GGAAACTTGGTCTCCATCAGG
haox2_SALK_102409_RP	Genotyping	GCTCGACTATTCCCCTGCTAC
LBb1.3	Genotyping	ATTTTGCCGATTTCGGAAC
SAIL_LB3	Genotyping	TAGCATCTGAATTTCATAACCAATCTCGATACAC
GK_o8474	Genotyping	ATAATAACGCTGCGGACATCTACATTTT
SALK_newLB1	Genotyping	TGGACCGCTTGCTGCAACTCTC
CAT2_LP	Genotyping	CCCAGAGGTACCTCTTCTTCTCCCATG
CAT2_RP	Genotyping	TCAGGGAACTTCATCCCATCGC
GOX1_F_CDS	qRT-PCR	TCTCATTGGCAGCTGAAGGA
GOX1_R_CDS	qRT-PCR	GAGTGTCCCATTCGGTGGTA
GOX2_F_CDS	qRT-PCR	TTTGCACTAGCTGCTGAAGGA
GOX2_R_CDS	qRT-PCR	ATAACCTGGGCAAATGGCGT
GOX1_F	qRT-PCR	AGAACAGCAGCAACACAGAAC
GOX1_R	qRT-PCR	CACTAGGCTTGGTTTGTGATCTGATA
GOX2_F	qRT-PCR	ATATCTCGAAAAACTTGTTTCTCCCTATAT
GOX2_R	qRT-PCR	ATCTATTGTACAAGCAATAAGAATAAACGG
EF1a_F	qRT-PCR	TCCGTCGGAGCTCAATTCTC
EF1a_R	qRT-PCR	AGGAAGCTCGAGTGCCAAGTAC
UBQ5_F	qRT-PCR	CTGCATTTCTATTTGGGAATTTTGTAA
UBQ5_R	qRT-PCR	ATCCAGAACGAAAGATGTTCAACATAG

Supplemental Table S5. Mutations enriched in line *238.3*.

Chromosome	Position	Reference base	Mutant	Coverage	Frequency	Region	AGI code	Position in gene	Position in codon	Reference amino acid	Mutant
3	4822263	G	A	65	0.9	CDS	*At3g14420*	263	2	G	E
3	7636562	G	A	40	0.89	intergenic					
3	6290758	G	A	60	0.86	intergenic					
3	3807077	G	A	54	0.84	intergenic					
3	6145283	G	A	43	0.83	five_prime_UTR	*At3g17940*				
4	8981113	G	A	5	0.83	intronic/noncoding	*At4g15780*				
3	6021032	G	A	50	0.82	CDS	*At3g17600*	9	3	V	V
3	436295	C	T	60	0.8	CDS	*At3g02260*	11022	3	E	E
3	3397230	G	A	37	0.8	intronic/noncoding	*At3g10845*				
3	6031593	G	A	51	0.8	CDS	*At3g17630*	2223	3	K	K

Supplemental Table S6. Data sources and sequence accessions.

Data source	Species	Accession
PLAZA 3.0 Dicots	*Arabidopsis thaliana*	AT3G14415
		AT3G14420
	Thellungiella parvula	TP3G12710
		TP3G12720
EnsemblPlants release 30	*Arabidopsis lyrata*	fgenesh2_kg.3__1563__AT3G14420.2
	Brassica rapa	Bra021556
		Bra021555
		Bra001550
		Bra001551
		Bra027338
		Bra027339
	Solanum lycopersicum	Solyc07g056540.2
	Solanum tuberosum	PGSC0003DMG400027654
Phytozome 9	*Capsella rubella*	Carubv10014001m.g
		Carubv10013998m.g
	Thellungiella halophila	Thhalv10020979m.g
		Thhalv10020982m.g