A novel allele in the *Arabidopsis thaliana* MACPF protein CAD1 results in deregulated immune signaling

Danalyn R. Holmes, Melissa Bredow, Kathrin Thor, Sydney A. Pascetta, Irina Sementchoukova, Kristen R. Siegel, Cyril Zipfel and Jacqueline Monaghan

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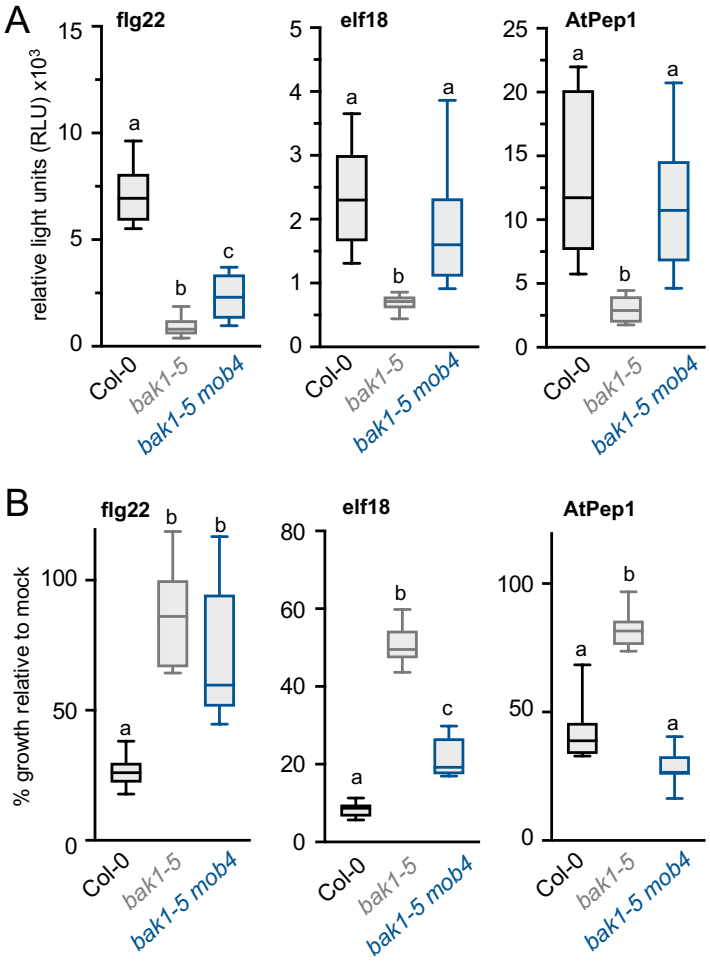
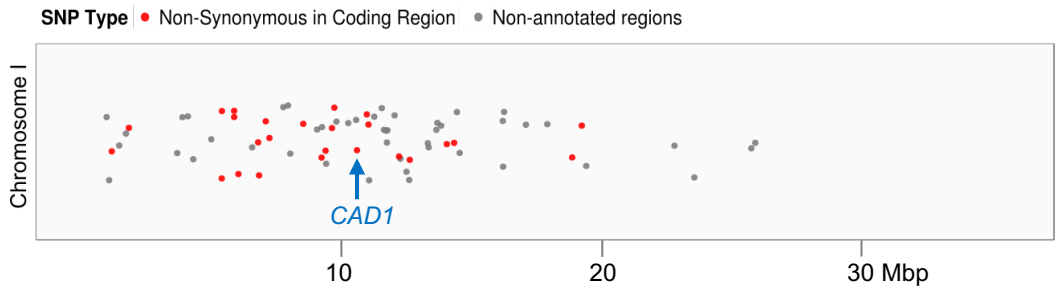


Figure S1: MAMP responsiveness is partially restored in *bak1-5 mob4*.
(A) ROS production in the indicated genotypes after treatment with 100 nM flg22, 100 nM elf18, or 1 µM AtPep1. Values represent total photon count (relative light units) over 40 min (n=8). **(B)** Seedling inhibition after 12 days of growth in 100 nM flg22, 100 nM elf18, or 1 µM AtPep1 in the indicated genotypes, shown as % growth (fresh weight) relative to growth in control MS media (n=12).
All experiments were repeated at least three times with similar results. Statistically significant (p<0.05) groups were analyzed by ANOVA followed by Tukey's posthoc test and are indicated by lower-case letters.

A



B

Chr	Pos	Ref	Alt	Allele_Freq	Change	Gene ID	Gene Name	Sanger BC
1	1116697	C	T	79.16	S/F	AT1G04210	Raf-related kinase	heterozygous
1	1763961	C	T	86.2	V/M	AT1G05840	Proteolysis	heterozygous
1	5271282	C	T	100	S/N	AT1G15310	SRP45	heterozygous
1	5276811	C	T	84.61	R/K	AT1G15340	MBD10	heterozygous
1	5740226	C	T	87.5	R/K	AT1G16780	Golgi proton pump	heterozygous
1	5741941	C	T	85.18	E/K	AT1G16780	Golgi proton pump	heterozygous
1	5901236	C	T	100	S/F	AT1G17255	Antisense gene	background
1	6646943	C	T	86.2	L/F	AT1G19230	RbohE	heterozygous
1	6681734	C	T	86.66	E/K	AT1G19330	Protein of unknown function	homozygous
1	6934331	C	T	85	T/I	AT1G19980	Cytomatrix protein related	heterozygous
1	7073500	T	G	75	T/P	AT1G20400	Protein of unknown function	not found
1	8351039	C	T	92.3	S/F	AT1G23550	SRO1	homozygous
1	9042744	C	T	95.23	E/K	AT1G26150	PERK10	homozygous
1	9194612	C	T	93.93	S/F	AT1G26610	Nucleic acid binding	homozygous
1	9437278	C	T	96.29	T/I	AT1G27170	TM receptor	homozygous
1	9525661	C	T	100	G/E	AT1G27430	GYF protein	homozygous
1	10381734	C	T	100	C/Y	AT1G29690	CAD1	homozygous
1	10752574	C	T	86.11	P/S	AT1G30420	ATP cassette	heterozygous
1	10815851	C	T	90	T/I	AT1G30530	UGT78D1	homozygous
1	11969678	C	T	75	E/K	AT1G33050	Protein of unknown function	homozygous
1	12375231	C	T	83.33	L/F	AT1G34040	Pyridoxal phosphate transferase	homozygous
1	13774328	C	T	75	A/V	AT1G36520	Transposable element gene	not tested
1	14051638	C	T	77.77	S/F	AT1G37030	Transposable element gene	not tested
1	18510852	C	T	76.19	T/I	AT1G49980	DNA repair	homozygous
1	18872523	C	T	76	H/Y	AT1G50920	GTP binding	homozygous
3	13080899	G	A	76	D/N	AT3G32091	Transposable element gene	background
3	13081217	G	A	87.5	A/T	AT3G32091	Transposable element gene	background
4	7211101	C	T	76.47	R/C	AT4G12030	BASS5/BAT5	background
5	5599873	G	A	83.33	G/S	AT5G17020	XPO1	not tested
5	9186001	G	A	83.33	G/R	AT5G26236	Transposable element gene	background
5	20881895	G	A	90.9	R/*	AT5G51410	LUC7	heterozygous
5	21236328	G	A	85	P/L	AT5G52290	SHOC1	heterozygous

Figure S2: Identification of single nucleotide polymorphisms in *bak1-5 mob4* by whole-genome sequencing of bulked segregants.
(A) CandiSNP output indicating the position of unique single nucleotide polymorphisms (SNPs) in bulked *bak1-5 mob4* mutants after a single cross to *bak1-5*. Red spots indicate SNPs that cause non-synonymous changes, while grey spots represent SNPs in regions that are not annotated as protein-coding. **(B)** Sequencing results of SNPs causing non-synonymous changes (red spots in A), indicating the Chromosome (Chr), Position (Pos), reference (Ref) and alternate (Alt) alleles, the % frequency the alternate allele was represented in Illumina reads (Allele_Freq), the expected amino acid change (Change), the gene ID, and the gene name. These SNPs were genotyped in individual recombinants isolated from a backcross (BC) by Sanger sequencing. The grey area indicates SNPs that were homozygous in each individual recombinant tested.

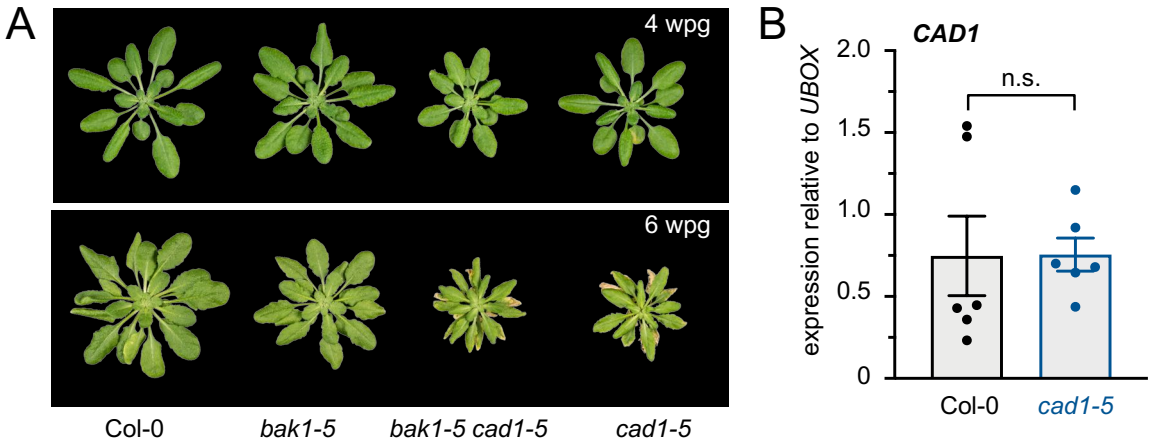


Figure S3: *cad1-5* is not suppressed by *bak1-5*.

(A) Plants were photographed after growth in a short-day chamber at 4 and 6 weeks post germination (wpg) as indicated. Genotypes were grown routinely over five years with similar phenotypes. **(B)** Real time quantitative reverse-transcription PCR of *CAD1* was performed and plotted relative to expression of *UBOX.* Values are means + standard error from six independent experiments. A paired Student's t-test indicates that there is no significant (n.s.) difference between *CAD1* expression in Col-0 and *cad1-5* (p=0.3543).

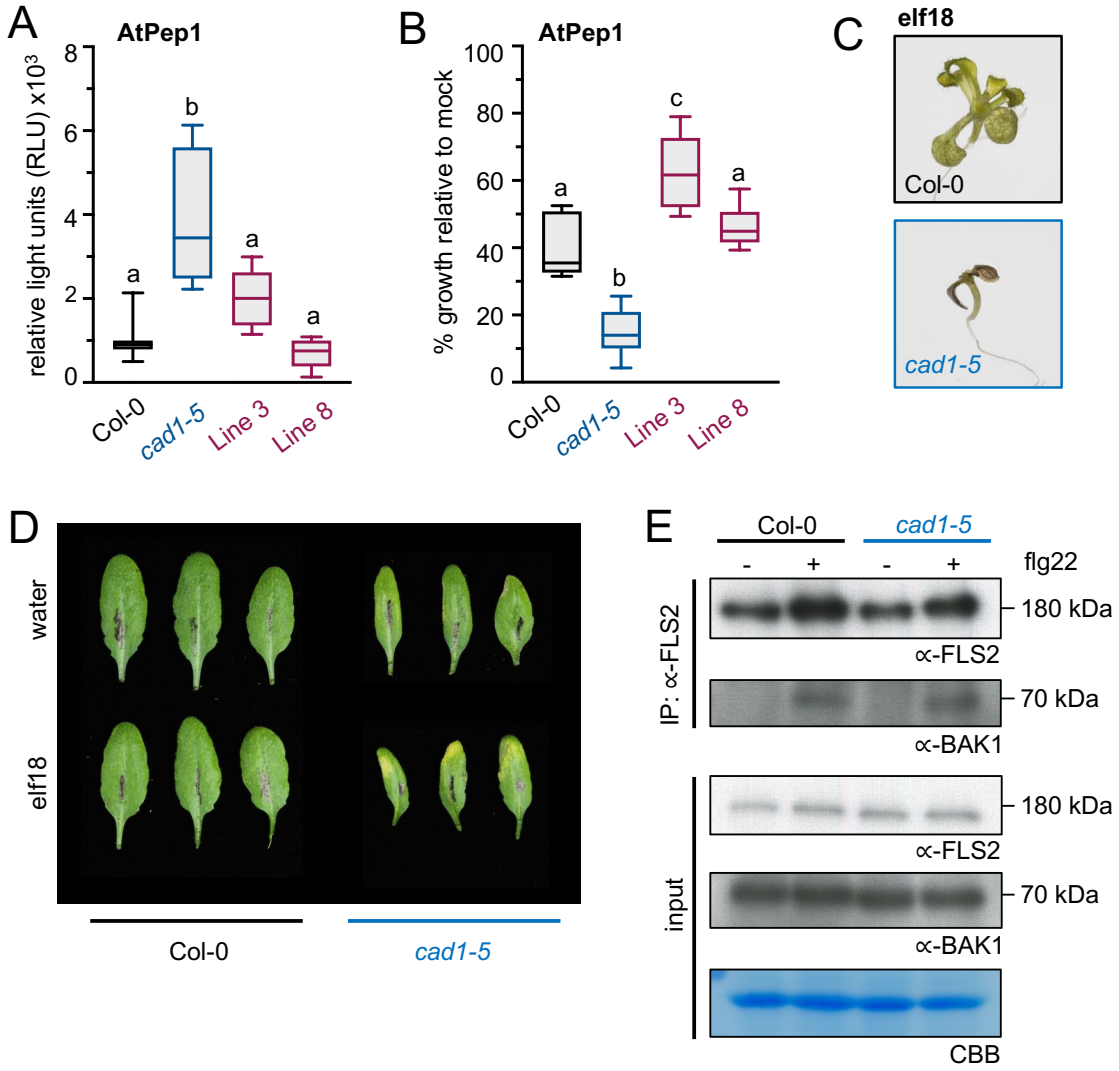


Figure S4: Enhanced MAMP-triggered responses in *cad1-5*.

(A) ROS production in Col-0, *cad1-5*, and two independent lines (Line 3 and Line 8) of *cad1-5/pCAD1:CAD1* after treatment with 1 µM AtPep1. Values represent total photon count (relative light units) over 40 min (n=6). **(B)** Seedling inhibition after 12 days of growth in 1 µM AtPep1 in the indicated genotypes, shown as % growth (fresh weight) relative to growth in control MS media (n=6). **(C)** Photograph of representative Col-0 and *cad1-5* seedlings after 12 days of growth in 100 nM elf18, indicating severe necrosis in *cad1-5*. **(D)** Five-week old plants were syringe-infiltrated with water or 1 µM elf18 and photographed after 24h. Induced cell death is observed in *cad1-5* following immune-induction. **(E)** Western blots following co-immunoprecipitation of the FLS2-BAK1 complex in Col-0 compared to *cad1-5*. All experiments were repeated at least three times with similar results. Statistically significant (p<0.05) groups were analyzed by ANOVA followed by Tukey's posthoc test and are indicated by lower-case letters.

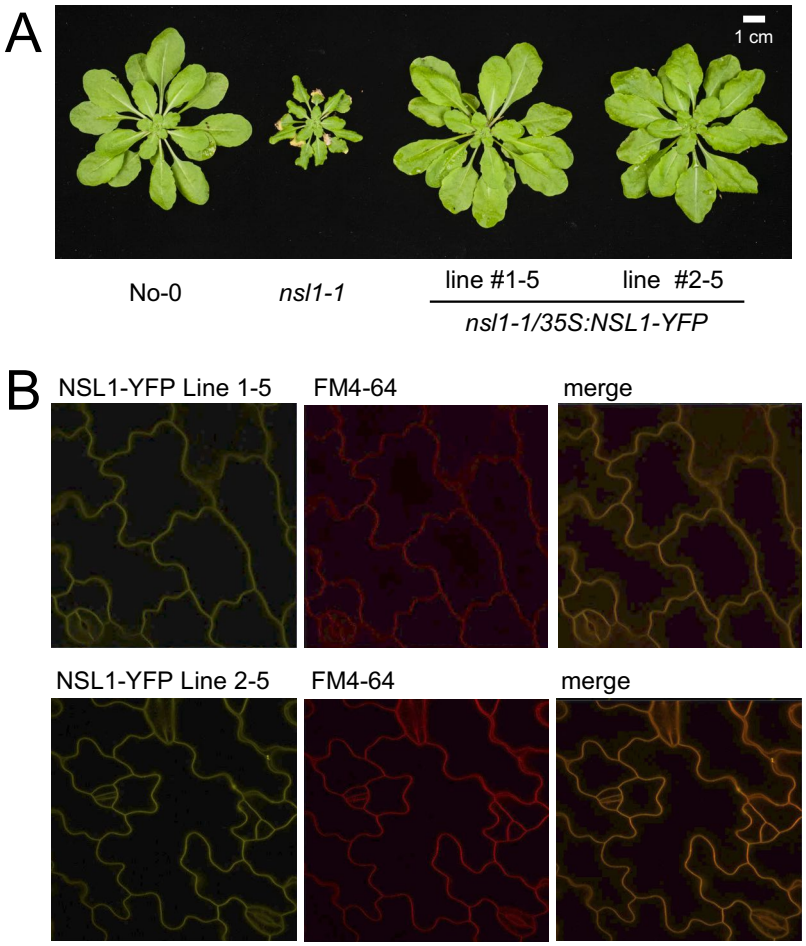


Figure S5: NSL1 localizes to the plasma membrane.

(A) Plants of the indicated genotypes (all in the No-0 ecotype) were grown in a short-day chamber and photographed 5 weeks post germination. Plants were grown several times over three years with similar phenotypes. **(B)** Confocal micrographs of NSL1-YFP expressed in cotyledon cells of two independent *nsl1-1/35S:cNSL1-YFP* transgenic lines. Seedlings were stained with the lipophilic dye FM4-64 prior to imaging to mark the plasma membrane. The merged image shows the overlay between the two channels. We observed clear plasma-membrane localization of NSL1-YFP in all four independent trials using these genotypes.

Figure S6: N-terminally tagged CAD1 confers a dominant-negative effect.

Plants were photographed after growth in a short-day chamber at 4 weeks post germination.

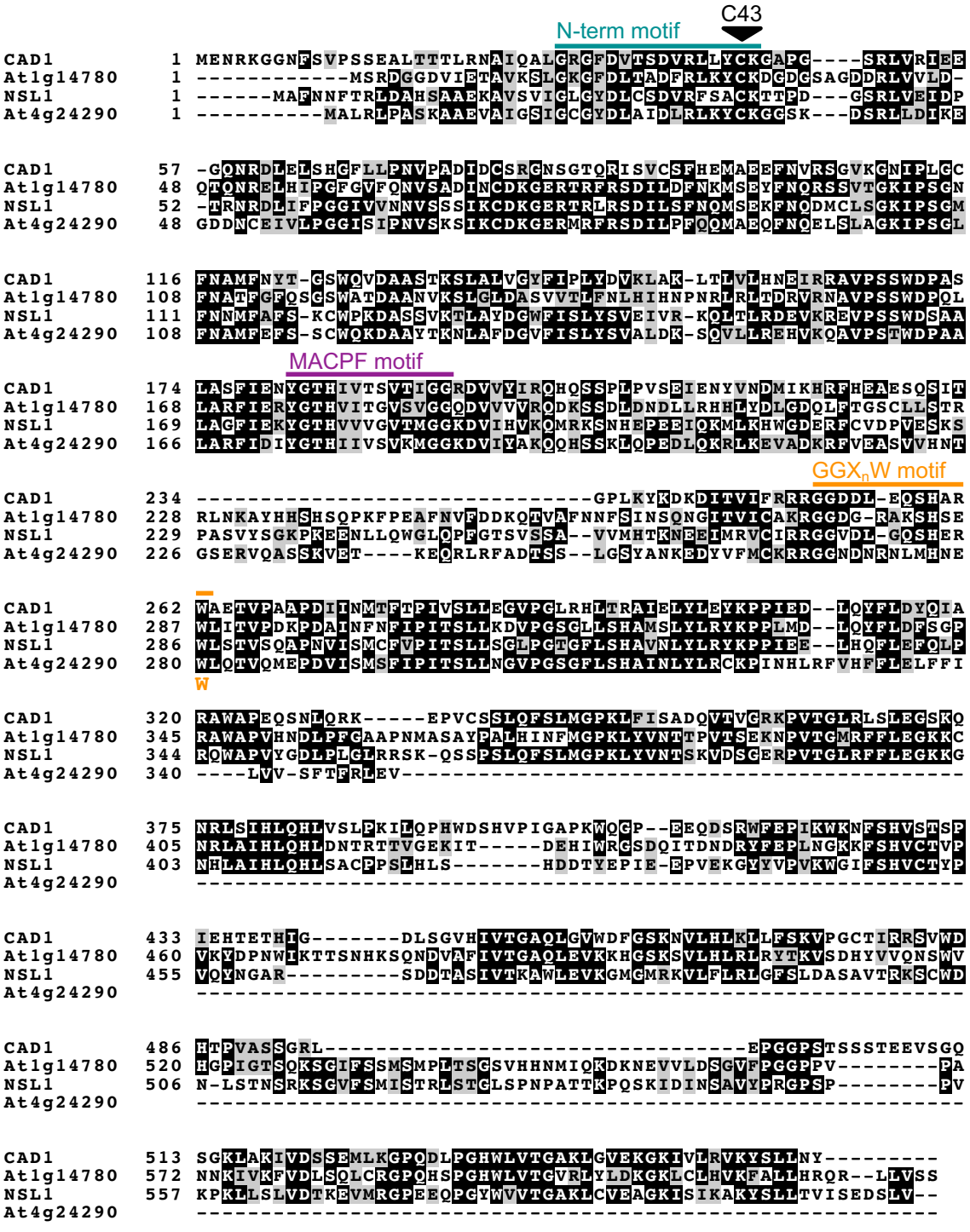


Figure S7: Multiple sequence alignment of Arabidopsis MACPF proteins.

Sequence alignment was performed using ClustalOmega and visualized with BoxShade. Identical residues are shown in black, similar residues in gray. The location of the conserved MACPF motif is shown in green; GGXnW motif is shown in blue; and the position of the *cad1-5* C43Y mutation is indicated by the orange arrow.

Supplementary Table

Table S1: Germplasm, primers, and constructs used in this study.

***Arabidopsis thaliana* germplasm**		
Seed line	**Primers used for genotyping (5'-3')**	
Col-0	-	-
No-0	-	-
bak1-5 (Schwessinger et al., 2011)	TTGGGATCTGCAAGAGGGCTTGCGTATTTACATGATCAGT	GAGGCGAGCAAGATCAAAAG
eds1-2 (Feys et al., 2005)	ACACAAGGGTGATGCGAGACA	GTGGAAACCAAATTTGACATTAG
ndr1-1 (Century et al., 1997)	GGCAACAAGCTATATGAAACC	GAATACGAGTAAATTCATCAA
cad1-5	GTGTTGGTGAGAGTCTAA	TAGACATGTCCAAAGTA
cad1-2 (GK_192A09)	CTTACGCCCAACAGTTACCTG	GATCAGACGTGCTGTTCCTTC
cad1-3 (GK_385H08)	TACCAAATAGCTCGAGCTTGG	TCAAACCGAACAGAACCAAAC
nsl1-1 (PSH_21828)	TCCAGGTTGTTCCTCTGGAC	GTTTCAACTTCCACGCCAAT
cad1-5 bak1-5	-	-
cad1-5 eds1-2	-	-
cad1-5 ndr1-1	-	-
SNP in *bak1-5 mob4*	**Primers used for mapping (5'-3')**	
Chr1_1116697	GCAGGCTCCCACCGTAA	CTGTCCAAGAGTATGACT
Chr1_1763961	AGTGACATTCGGAAACC	CAAAAGTAAACATGACA
Chr1_5271282	GATGCAGACCAATATT	GCAAACTGTTGAGATCT
Chr1_5276811	TTGACCCTCCTTCCCAGCCTC	CTCTTAGATTGTGCCTTGT
Chr1_5740226	ATGAGCCAGCAGGTATGA	CACCAGTTACTGCAGCTTTGT
Chr1_5741941	TATCTTCTTATACCAAG	CTGCTAGTTTGACCACAGT
Chr1_5901236	CTCGAGCTTTCTCATAA	ACTATGCTGCCGTGATCTTGCTG
Chr1_6646943	GTCTGGAAGTTTCTAGAG	TGCGTTAAAGCCAGTCAACCG
Chr1_6681734	GAGTCAGATGATAGAGAC	GAGTAACTTCACCATATTC
Chr1_6934331	GTATCGAGAGTGAATTCACT	GCTCCATTAGATGGAGACTTC
Chr1_7073500	ACGACCCGAGTAGTAATGCT	GCAGTCAGCGACGAAGCCAAAGAT
Chr1_8351039	CTCGCACTTTCTTCTAGACTC	GAGATGATTCTTTGAATCT
Chr1_9042744	GGGGAGAAGATTCTGGTG	GTTAGAATCTGGTCTTGG
Chr1_9194612	AAGATGTTCAATGTGAAGAAT	GCTTTCTTAACCATTG

Chr1_9437278	CAGGTGGATGAGAATTTG	CCACCGCGCTTCCGTTGAT
Chr1_9525661	TCTGTGGTTAGCAATGT	ACAGACTGTTTCCACC
Chr1_10381734 (*cad1-5*)	GTGTTGGTGAGAGTCTAA	TAGACATGTCCAAAGTA
Chr1_10752574	ATTCCTCTTGCAGTTTAATAG	TGATATCAACGCTTGAGGTTTC
Chr1_10815851	AGATGTGAGTATGGAAG	GACTCCATTATCCATCATC
Chr1_11969678	CATATCGCTTGTAGATGAACT	TTCTCATAAGAGTCTGGCTTCTGG
Chr1_12375231	TTGGAGCTGGAGCCACA	GTCACCCGAATCTTGA
Chr1_13774328	GAATGTCTTGAGAGGCGATCT	CTATGCCTTAGATGAATGCAGA
Chr1_14051638	GCATTGATCCGGCTACATTG	GCAGTCATTCAAGGTCGATG
Chr1_18510852	GCATTACTTGTGAAGTCAAGT	TCTTCATATTCGACAACATCCTTC
Chr1_18872523	TCGGATGAGCAGCTGAAGCAGC	TAGATTCGTCAACTCAGGA
Chr3_1308089/1217	TCTCAAGATTACCGTAATCAG	ATCCAACTCTGCATCTCCTTGT
Chr4_7211101	ATGATAATTGTCCAATCGA	GTTGATCCAAACCTTGGCTTGAAC
Chr5_5599873	GAAGACAGTTGTGAACAAGTTG	GCACTGTACGTATCACAACTTG
Chr5_9186001	GTGAGCCTGAAGGAGATGTT	TGGAATCCCTGTCTGACACAATA
Chr5_20881895	AATCTTGTTTCATTTGAGC	TAATACCTACCTCAACGCT
Chr5_21236328	CACACCACAAATTTCGA	CAGTCTGAGCTCCCAAGACGG

Seed line	**Construct used**	***In planta* resistance marker**
bak1-5 mob4/ pCAD1:gCAD1 line 7	pGWB1 - *pCAD1:gCAD1*	Kanamycin
bak1-5 mob4/ pCAD1:gCAD1 line 9		
cad1-5/ pCAD1:gCAD1 line 3		
cad1-5/ pCAD1:gCAD1 line 8		
cad1-5/ pCAD1:gCAD1-GFP line 4	pGWB4 - *pCAD1:gCAD1-GFP*	Kanamycin
cad1-5/ pCAD1:gCAD1-GFP line 5		
Col-0/ 35S:GFP-gCAD1 line 10	pGWB6 - *35S:GFP-gCAD1*	Kanamycin
Col-0/ 35S:GFP-gCAD1 line 5		
Col-0/ 35S:gCAD1 line 5	pGWB2 - *35S:gCAD1*	Kanamycin
Col-0/ 35S:gCAD1 line 6		
nsl1-1/ 35S:NSL1-YFP line 1	pXCSG - *35S:NSL1-YFP*	Basta
nsl1-1/ 35S:NSL1-YFP line 2		

Gene constructs		
Entry vectors	**Primers used for cloning (5'-3')**	
pENTR - *cCAD1* (no promoter, no stop codon)	CACCATGGAGAATCGTAAAGGAGG	ATAATTTAGCAACGAATACTT
pENTR - *cCAD1* (no promoter, native stop codon)	CACCATGGAGAATCGTAAAGGAGG	TCAATAATTTAGCAACGAATA
pENTR - *gCAD1* (native promoter, native stop codon)	CACCCATCCTCTAATTTGAAGCAT	TCAATAATTTAGCAACGAATA
pENTR - *gCAD1* (native promoter, no stop codon)	CACCCATCCTCTAATTTGAAGCAT	ATAATTTAGCAACGAATACTT
pENTR - *gCAD1* (no promoter, no stop codon)	CACCATGGAGAATCGTAAAGGAGG	ATAATTTAGCAACGAATACTT
pENTR - *gCAD1-C43Y* (no promoter, no stop codon) *amplified from *cad1-5* gDNA	CACCATGGAGAATCGTAAAGGAGG	ATAATTTAGCAACGAATACTT
pENTR - *cNSL1* (no promoter, no stop codon)	CACCATGGCCTTTAACAATTTCAC	GACCAGTGAGTCTTCCGATA
Destination vectors	**Entry vector**	**Backbone**
pGWB1 - *pCAD1:gCAD1*	pENTR - *gCAD1* (native promoter, native stop codon)	pGWB1
pGWB4 - *pCAD1:gCAD1-GFP*	pENTR - *gCAD1* (native promoter, no stop codon)	pGWB4
pGWB6 - *35S:GFP-gCAD1*	pENTR - *cCAD1* (no promoter, native stop codon)	pGWB6
pGWB2 - *35S:gCAD1*	pENTR - *cCAD1* (no promoter, native stop codon)	pGWB2
pXCSG - *35S:gCAD1-YFP*	pENTR - *gCAD1* (no promoter, no stop codon)	pXCSG
pXCSG - *35S:gCAD1-C43Y-YFP*	pENTR - *gCAD1-C43Y* (no promoter, no stop codon)	pXCSG
pXCSG - *35S:NSL1-YFP*	pENTR - *cNSL1* (no promoter, no stop codon)	pXCSG

Primers used for quantitative real-time PCR		
Target gene	**Primers used for qPCR (5'-3')**	
PR1	GTAGGTGCTCTTGTTCTTCCC	CACATAATTCCCACGAGGATC
UBOX	TGCGCTGCCAGATAATACACTATT	TGCTGCCCAACATCAGGTT
CAD1	GCTTCCATGAGATGGCAGAA	CAATAAAGCTAGCTAGGGAAGC